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(57) Abstract

The structure and function of a regulatory gene, SCARECROW (SCR), is described. The SCR gene is expressed specifically in root progenitor tissues of embryos, and in roots and stems of seedlings and plants. SCR expression controls cell division of certain cell types in roots and affects the organization of root tissues, and affects gravitropism of aerial structures. The invention relates to the SCARECROW (SCR) gene, SCR gene products, (including but not limited to transcriptional products such as mRNAs, antisense, and ribozyme molecules, and translational products such as the SCR protein, polypeptides, peptides and fusion proteins related thereto), antibodies to the SCR product, SCR promoters and regulatory regions and the use of the foregoing to improve agronomically valuable plants.

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SCARECROW GENE, PROMOTER AND USES THEREOF

This application is a continuation-in-part of co-pending Application No. 08/638,617, filed April 26, 1996, 5 the disclosure of which is incorporated by reference in its entirety.

This invention was made with government support under grant number: GM43778 awarded by the National

10 Institute of Health. The government may have certain rights in the invention.

1. <u>INTRODUCTION</u>

The present invention generally relates to the

15 SCARECROW (SCR) gene family and their promoters. The
invention more particularly relates to ectopic expression of
members of the SCARECROW gene family in transgenic plants to
artificially modify plant structures. The invention also
relates to utilization of SCARECROW promoter for tissue and
20 organ specific expression of heterologous gene products.

2. BACKGROUND OF THE INVENTION

Asymmetric cell divisions, in which a cell divides to give two daughters with different fates, play an important role in the development of all multicellular organisms. In plants, because there is no cell migration, the regulation of asymmetric cell divisions is of heightened importance in determining organ morphology. In contrast to animal embryogenesis, most plant organs are not formed during embryogenesis. Rather, cells that form the apical meristems are set aside at the shoot and root poles. These reservoirs of stem cells are considered to be the source of all postembryonic organ development in plants. A fundamental question in developmental biology is how meristems function 35 to generat plant organs.

2.1. ROOT DEVELOPMENT

Root organization is established during embryogen sis. This organization is propagated during postembryonic development by the root meristem. Following 5 germination, the development of the postembryonic root is a continuous process, a series of initials or stem cells continuously divide to perpetuate the pattern established in the embryonic root (Steeves & Sussex, 1972, Patterns in Plant Development, Englewood Cliffs, NJ: Prentice-Hall, Inc.).

- Due to the organization of the Arabidopsis root it is possible to follow the fate of cells from the meristem to maturity and identify the progenitors of each cell type (Dolan et al., 1993, Development 119:71-84). The Arabidopsis root is a relatively simple and well characterized organ.
- 15 The radial organization of the mature tissues in the Arabidopsis root has been likened to tree rings with the epidermis, cortex, endodermis and pericycle forming radially symmetric cell layers that surround the vascular cylinder (FIG. 1A). See also Dolan et al., 1993, Development
- 20 119:71-84. These mature tissues are derived from four sets of stem cells or initials: i) the columella root cap initial; ii) the pericycle/vascular initial; iii) the epidermal/lateral root cap initial; and iv) the cortex/endodermal initial (Dolan et al., 1993, Development
- 25 119:71-84). It has been shown that these initials undergo asymmetric divisions (Scheres et al., 1995, Development 121:53-62). The cortex/endodermal initial, for example, first divides anticlinally (in a transverse orientation) (FIG. 1B). This asymmetric division produces another initial
- 30 and a daughter cell. The daughter cell, in turn, expands and then divides periclinally (in the longitudinal orientation) (FIG. 1B). This second asymmetric division produces the progenitors of the endodermis and the cortex cell lineages (FIG. 1B).

2.2. GENES REGULATING ROOT STRUCTURE

Mutations that disrupt the asymmetric divisions of the cortex/endodermal initial have be n identified and characterized (Benfey et al., 1993, Development 119:57-70; 5 Scheres et al., 1995, Development 121:53-62). short-root (shr) and scarecrow (scr) mutants are missing a cell layer between the epidermis and the pericycle. In both types of mutants the cortex/endodermal initial divides anticlinally, but the subsequent periclinal division that increases the 10 number of cell layers does not take place (Benfey et al., 1993, Development 119:57-70; Scheres et al., 1995, Development 121:53-62). The defect is first apparent in the embryo and it extends throughout the entire embryonic axis which includes the embryonic root and hypocotyl (Scheres et 15 al., 1995, Development 121:53-62). This is also true for the other radial organization mutants characterized to date, suggesting that radial patterning that occurs during embryonic development may influence the post-embryonic pattern generated by the meristematic initials (Scheres et 20 al., 1995, Development 121:53-62).

Characterization of the mutant cell layer in shr indicated that two endodermal-specific markers were absent (Benfey et al., 1993, Development 119:57-70). This provided evidence that the wild-type SHR gene may be involved in 25 specification of endodermis identity.

2.3. GEOTROPISM

In plants, the capacity for gravitropism has been correlated with the presence of amyloplast sedimentation.

30 See, e.g., Volkmann and Sievers, 1979, Encyclopedia Plant Physiol., N.S. vol 7, pp. 573-600; Sack, 1991, Intern. Rev. Cytol. 127:193-252; Björkmann, 1992, Adv. Space Res. 12:195-201; Poff et al., in The Physiology of Tropisms, Meyerowitz & Somerville (eds); Cold Spring Harbor Laboratory Press,

35 Plainview, NY (1994) pp. 639-664; Barl w, 1995, Plant Cell Environ. 18:951-962. Amyloplast s dim ntation only occurs in cells in specific locations at distinct developmental stages.

That is, wh n and wh r s dim ntation occurs is precisely regulated (Sack, 1991, Int rn. Rev. Cytol. 127:193-252). In roots, amyloplast s dimentation only occurs in the c ntral (columella) cells of the rootcap; as these cells mature into 5 peripheral cap cells, the amyloplasts no longer sediment (Sack & Kiss, 1989, Amer. J. Bot. 76:454-464; Sievers & Braun, in The Root Cap: Structure and Function, Wassail et al. (eds.), New York: M. Dekker (1996) pp. 31-49). of many plants, including Arabidopsis, amyloplast 10 sedimentation occurs in the starch sheath (endodermis) especially in elongating regions of the stem (von Guttenberg, Die Physiologischen Scheiden, Handbuch der Pflanzenanatomie; K. Linsbauer (ed.), Berlin: Gebruder Borntraeger, vol. 5 (1943) p. 217; Sack, 1987, Can. J. Bot. 65:1514-1519; Sack, 15 1991, Intern. Rev. Cytol. 127:193-252; Caspar & Pickard, 1989, Planta 177:185-197; Volkmann et al., 1993, J. Pl.

Physiol. 142:710-6). Gravitropic mutants have been studied for evidence that proves the role of amyloplast sedimentation in gravity 20 sensing. However, many gravitropic mutations affect downstream events such as auxin sensitivity or metabolism (Masson, 1995, BioEssays 17:119-127). Other mutations seem to affect gene products that process information from gravity sensing. For example, the lazy mutants of higher plants and 25 comparable mutants in mosses can clearly sense and respond to gravity, but the mutations reverse the normal polarity of the gravitropic response (Gaiser & Lomax, 1993, Plant Physiol. 102:339-344; Jenkins et al., 1986, Plant Cell Environ 9:637-644). Other mutations appear to affect gravitropism of 30 specific organs. For example, sgr mutants have defective shoot gravitropism (Fukaki et al., 1996, Plant Physiol. 110:933-943; Fukaki et al., 1996, Plant Physiol. 110:945-955; Fukaki et al., 1996, Plant Res. 109:129-137).

Citation or identification of any reference herein 35 shall not be construed as an admission that such ref rence is available as prior art to the present invention.

3. SUMMARY OF THE INVENTION

Th structure and function of a regulatory g ne, SCARECROW (SCR), is described. The SCR g ne is expr ssed specifically in root progenitor tissues of embryos, and in 5 certain tissues of roots and stems. SCR expression controls cell division of certain cell types in roots, and affects the organization of root and stem. The invention relates to the SCARECROW (SCR) gene (which encompasses the Arabidopsis SCR gene and its orthologs and paralogs), SCR gene products,

10 (including but not limited to transcriptional products such as mRNAs, antisense and ribozyme molecules, and translational products such as the SCR protein, polypeptides, peptides and fusion proteins related thereto), antibodies to SCR gene products, SCR regulatory regions and the use of the foregoing 15 to improve agronomically valuable plants.

The invention is based, in part, on the discovery, identification and cloning of the gene responsible for the scarecrow phenotype. In contrast to the prevailing view that the SCR gene was likely to be involved in the specification of endodermis, the inventors have determined that the mutant cell layer in roots of scr mutants has differentiated characteristics of both cortex and endodermis. This is consistent with a role for SCR in the regulation of the asymmetric cell division rather than in specification of the identity of either cortex or endodermis. The inventors have also determined that SCR expression affects the gravitropism of plant aerial structures such as the stem.

One aspect of the invention relates to the heterologous expression of SCR genes and related nucleotide 30 sequences, and specifically the Arabidopsis SCR genes, in stably transformed higher plant species. Modulation of SCR expression levels can be used to advantageously modify root and aerial structures of transgenic plants and enhance the agronomic properties of such plants.

Anoth r aspect of the invention r lates to the us of promoters of SCR gens, and specifically the use f Arabidopsis SCR promoter to control the expression of protein

and RNA products in plants. Plant SCR promot rs have a variety of uses, including but not limited to xpr ssing het rologous g nes in the mbryo, ro t, root nodul, and st m of transformed plants.

- The invention is illustrated by working examples described infra which demonstrate the isolation of the Arabidopsis SCR gene using insertion mutagenesis. More specifically, T-DNA tagging of genomic and cDNA clones of the Arabidopsis SCR gene are described. Additional working examples include the isolation of SCR sequences from plant genomes using PCR amplification in combination with screening of genomic libraries, and heterologous gene expression in transgenic plants using SCR promoter expression constructs.
- Structural analysis of the deduced amino acid

 15 sequence of Arabidopsis SCR protein indicates that SCR
 encodes a transcription factor. Northern analysis, in situ
 hybridization analysis and enhancer trap analysis show highly
 localized expression of Arabidopsis SCR in embryos and roots.
 Genetic analysis shows SCR expression also affects
- 20 gravitropism of aerial structures (e.g., stems). This indicates that SCR is also expressed in those structures.

Computer analysis of the deduced amino acid sequence of Arabidopsis SCR protein with those of Expressed Sequence Tag (EST) sequences in GenBank reveals the existence 25 of at least thirteen SCR genes in Arabidopsis, one SCR gene in maize, four SCR genes in rice, and one SCR gene in Brassica. A further aspect of the invention relates to the use of such EST sequences to obtain larger and/or complete clones of the corresponding SCR gene.

The various embodiments of the claimed invention presented herein are by the way of illustration and are not meant to limit the invention.

3.1. DEFINITIONS

As used herein, the terms list d below will have the meanings indicated.

35S cauliflower mosaic virus promoter for the 355 transcript

compl mentary DNA

cis-regulatory

5 element A promoter sequence 5' upstream of the TATA box that confers specific regulatory response to a promoter containing such an element. A promoter may contain one or more cis-

regulatory elements, each responsible for a particular regulatory response

10 coding

CDNA

sequence sequence that encodes a complete or partial gene product (e.g., a complete protein or a

fragment thereof)

DNA deoxyribonucleic acid

EST expression tagged

15 functional

a functional portion of a promoter is any portion portion of a promoter that is capable of causing transcription of a linked gene

sequence, e.g., a truncated promoter

20 gene fusion

a gene construct comprising a promoter

operably linked to a heterologous gene, wherein said promoter controls the transcription of the heterologous gene

gene

25 product the RNA or protein encoded by a gene sequence

gene

35

sequence = sequence that encodes a complete gene product

(e.g., a complete protein)

GUS $1,3-\beta$ -Glucuronidase

30 **GDNA** genomic DNA

heterologous

gene In the context of gene constructs, a heterologous gene means that the gene is

linked to a promoter that said gene is not naturally linked to. The heterologous gene may or may not b from the organism

contributing said pr moter. The heterologous g n may ncode m ssenger RNA (mRNA),

antisens RNA or ribozymes

homologous

promoter = a nativ promoter of a g n that sel ctively

hybridiz s to the sequenc f a SCR g ne

described her in

mRNA = messenger RNA

operably

15

linked = A linkage between a promoter and gene sequence such that the transcription of said gene sequence is controlled by said promoter

ortholog = related gene in a different plant (e.g., maize ZCARECROW gene is an ortholog of the Arabidopsis SCR gene)

paralog = related gene in the same plant (e.g., Arabidopsis SRPal is a paralog of Arabidopsis SCR gene)

RNA = ribonucleic acid

RNase = ribonuclease

SCR = SCARECROW gene or gene product, encompasses (italic) SCR and ZCR genes and their orthologs and paralogs

20 SCR = SCARECROW protein

scr = scarecrow mutant (e.g., scrl)
(lower case)

ZCR = maize ZCARECROW gene, a paralog of, for example, the Arabidopsis SCR gene

SCR protein means a protein containing sequences or a domain substantially similar to one or more motifs (i.e., Motif I-VI), preferably MOTIF III (VHIID), of Arabidopsis SCR protein as shown in FIGS. 13A-F and FIGS. 15A-S. SCR proteins include SCR ortholog and paralog proteins having the structure and activities described herein.

SCR polypeptides and peptides include deleted or truncated forms of the SCR protein, and fragments corresponding to the SCR motifs described herein.

SCR fusion proteins encompass proteins in which the SCR protein or an SCR polypeptide or peptide is fused to a heterolog us protein, polypeptide or peptide.

SCR gene, nucleotides or coding sequences means nucl otides, e.g., gDNA or cDNA encoding SCR prot in, SCR polypeptides or peptides, or SCR fusion proteins.

SCR gene products include transcriptional products 5 such as mRNAs, antisense and ribozyme molecules, as well as translational products of the SCR nucleotides described herein including but not limited to the SCR protein, polypeptides, peptides and/or SCR fusion proteins.

SCR promoter means the regulatory region native to 10 the SCR gene in a variety of species, which promotes the organ and tissue specific pattern of SCR expression described herein.

4. BRIEF DESCRIPTION OF THE FIGURES

- FIGS. 1A-B. Schematic of Arabidopsis root anatomy. FIG. 1A. Transverse section showing the four tissues, epidermis, cortex, endodermis and pericycle that surround the vascular tissue. In the longitudinal section, the epidermal/lateral root cap initials and the cortex/endodermal
- 20 initials are shown at the base of their respective cell files. FIG. 1B. Schematic of division pattern of the cortex/endodermal initial. The initial expands then divides anticlinally to reproduce itself and a daughter cell. The daughter then divides periclinally to produce the progenitors
- 25 of the endodermis and cortex cell lineages. Abbreviations: C, cortex; Da, daughter cell; E, endodermis; In, initial.

FIGS. 2A-F. Phenotype of scr mutant plants.

IG. 2A. Shown left to right are 12-day scr-2, scr-1 and

- FIG. 2A. Shown left to right are 12-day scr-2, scr-1 and wild-type seedlings grown vertically on nutrient agar medium.
- 30 FIG. 2B. 21-day scr-2 mutant plants in soil. FIG. 2C. Transverse section through primary root of 7-day scr-2. FIG. 2D. Transverse section through primary root of 7-day wild-type (WT). FIG. 2E. Transverse section through lateral root of 12-day scr-1 mutant seedling. FIG. 2F. Transverse
- 35 section through root regenerated from scr-1 callus. Bar, 50 μ m. Abbreviations: C, cortex; En, endod rmis; Ep, pidermis; H, mutant cell layer; P, pericycle; V, vascular tissue.

FIGS. 3A-F. Charact rization of the c llular identity of the mutant c 11 lay r. FIG. 3A. Endodermisspecific Casparian band staining of transverse sections through the primary root of 7-day scr-1 mutant. (Note: the 5 histochemical stain also reveals xylem cells in the vascular cylinder.) FIG. 3B. Casparian band staining of transverse sections through the primary root of 7-day wild-type (WT). Immunostaining with the endodermis (and a subset of vascular tissue) specific JIM13 monoclonal antibodies on 10 transverse root sections of scr-2 mutant. FIG. 3D. Immunostaining with JIM13 monoclonal antibodies on transverse root sections of WT. FIG. 3E. Immunostaining with the JIM7 monoclonal antibody that stains all cell walls on transverse root sections of scr-2 mutant. FIG. 3F. Immunostaining with 15 JIM7 monoclonal antibodies on transverse root sections of WT. Bar, 25 µm. Abbreviations are same as those for description of FIGS. 2A-2F and: Ca, casparian strip.

FIGS. 4A-F. Immunostaining. FIG. 4A.

Immunostaining with the cortex (and epidermis) specific CCRC-20 M2 monoclonal antibodies on transverse root sections of scr-1 mutant. FIG. 4B. Immunostaining with CCRC-M2 antibodies on transverse root sections of scr-2 mutant. FIG. 3C. Immunostaining with CCRC-M2 antibodies on transverse root sections of wild-type (WT). FIG. 4D. Immunostaining with the CCRC-M1 monoclonal antibodies (specific to a cell wall epitope found on all cells) on transverse root sections of scr-1. FIG. 4E. Immunostaining with CCRC-M1 antibodies on transverse root sections of scr-2. FIG. 4F. Immunostaining with CCRC-M1 antibodies on transverse root sections of WT.
30 Bar, 30 μm. Abbreviations are same as those for description

30 Bar, 30 μm . Abbreviations are same as those for description of FIGS. 2A-2F.

FIG. 5A-E. Structure of the Arabidopsis SCARECROW gene. FIG. 5A. Nucleic acid sequence and deduced amino acid sequence of the Arabidopsis SCR genomic region (SEQ ID NO:1)

35 and (SEQ ID NO:2), respectively. Regulatory sequences including: (i) TATA box, (ii) ATG start codon, and (iii) potential polyad nylation sequence are underlin d. Within

th deduc d amino acid sequence homopolym ric rep ats are underlined. FIG. 5B. Sch matic diagram of gen mic clone indicating possible functional motifs, T-DNA insertion sites and subclones used as probes. Abbreviations: Q,S,P,T, region 5 with homopolymeric repeats of these amino acids; b, region with similarity to the basic region of bZIP factors; I and II, regions with leucine heptad repeats; E, acidic region. FIG. 5C. Comparison of the charged region found in Arabidopsis SCR protein with that found in bZIP transcription 10 factors, SCR bZIP-like domain (SEQ ID NO:3), GCN4 (SEQ ID NO:4), TGA1 (SEQ ID NO:5), C-Fos (SEQ ID NO:6), C-JUN (SEQ ID NO:7), CREB (SEQ ID NO:8), Opaque-2 (SEQ ID NO:9), OBF2 (SEQ ID NO:10), RAF-1 (SEQ ID NO:11). FIG. 5D. Translations of EST clones encoding putative peptide having similarities to 15 the VHIID domain region of Arabidopsis SCR protein (SEQ ID NO:12), F13896 (SEQ ID NO:13), Z37192 (SEQ ID NO:14), and Z25645 (SEQ ID NO:15) are from Arabidopsis, T18310 (SEQ ID NO:17) is from maize and D41474 (SEQ ID NO:16) is from rice. FIG. 5E. The deduced amino acid sequence of the Arabidopsis 20 SCARECROW gene (SEQ ID NO:2).

FIGS. 6A-B. Expression of the Arabidopsis

SCARECROW gene. FIG. 6A. Northern blot of total RNA from
wild-type siliques (Si), roots (R), leaves (L) and whole
seedlings (Sd) hybridized with Arabidopsis SCR probe a and
25 with a probe from the Arabidopsis glutamine dehydrogenase
(GDH) gene (Melo-Oliveira et al., 1996, Proc. Natl. Acad.
Sci. USA 93:4718-4723) as a control for RNA integrity. (GDH
expression is lower in siliques than in vegetative tissues.)
The 1.6 kb band corresponds to the GDH gene and the
30 approximately 2.5 kb band corresponds to SCR. Ribosomal RNA
is shown as a loading control. FIG. 6B. Northern blot of
Arabidopsis wild-type, scr-1 and scr-2 total RNA, probed with
Arabidopsis SCR probe "a" corresponding to a cDNA sequence
shown in FIG. 5B, and with the GDH probe. In scr-2 mutant
35 additional bands f 4.1 kb and 5.0 kb were detected.

FIGS. 7A-G. In situ hybridization and enhanc r trap analyses of Arabidopsis SCR xpression. FIG. 7A. SCR

RNA xpression detected by in situ hybridization of SCR antisense prob to a 1 ngitudinal s ction through the root meristem. FIG. 7B. In situ hybridization of SCR antis nse probe to a transverse section in the meristematic region.

- 5 FIG. 7C. In situ hybridization of SCR antisense probe to late torpedo stage embryo. FIG. 7D. Negative control in situ hybridization using a SCR sense probe to a longitudinal section through the root meristem. FIG. 7E. GUS expression in a whole mount in the enhancer trap line, ET199 in primary
- 10 root tip. FIG. 7F. GUS expression in the ET199 line in transverse root section in the meristematic region. FIG. 7G. GUS expression in ET199 detected in a section through the root meristem. GUS expression is observed in the cortex/endodermal initial, and in the first cell in the
- 15 endodermal cell lineage but not in the first cell of the cortex lineage. Expression in two endodermal layers is observed higher up in the root because the section was not median at that point. Bar, 50 μ m. Abbreviations are same as those in the description of FIGS. 2A-2F.
- 20 FIG. 8. Partial nucleotide sequence (SEQ ID NO:18) and deduced amino acid sequence (SEQ ID NO:19) of the Arabidopsis SRPa4 gene.
- FIG. 9. Partial nucleotide sequence (SEQ ID NO:20) and deduced amino acid sequence (SEQ ID NO:21) of the 25 Arabidopsis SRPa3 gene.
 - FIG. 10. Partial nucleotide sequence (SEQ ID NO:22) of the Arabidopsis SRPa1 gene.
- FIG. 11A. Nucleotide sequence (SEQ ID NO:24) and deduced amino acid sequence (SEQ ID NO:25) of the maize Zm-30 Scl1 fragment.
 - FIG. 11B. Partial nucleotide sequence (SEQ ID NO:25) and deduced amino acid sequence (SEQ ID NO:26) of the maize SRPm1 gene (Zm-Scl2).
- FIG. 12A-B. Nucleotide sequence of rice SRP03 EST 35 clone. FIG. 12A. Sequence of 5' end f EST clon (SEQ ID NO:28). FIG. 12B. Sequence of 3' nd of EST cl n (SEQ ID NO:29).

FIGS. 13A-F. C mparison of the amino acid sequence f m mbers of th SCARECROW family of g n s. C nserved Motifs I through VI are indicated by dash d line above the aligned sequences. Consensus sequences are shown in bold.

5 See Table 1 for the identity and sequence identifier number of each of the sequences shown in this Figure. Hu-scr-1 = Human SCR paralog (SEQ ID NO:40).

FIG. 14. Restriction map of the approximately 8.8 kb Eco RI insert DNA of lambda clone, t643, containing the 10 Arabidopsis SCR gene. The locations of the approximately 5.6 kb HindIII-SacI fragment subcloned in plasmid LIG 1-3/SAC+MoB, 1SAC, and the SCR coding region are indicated below the restriction map. The location of the translational initiation site of the SCR gene is at the Nco I site at the 15 left end of the indicated coding region. The SCR coding sequence begins at the translation initiation site and extends approximately 1955 nucleotides to its right. E. coli DH5α containing plasmid pLIG1-3/SAC+MoB, 1SAC, has the ATCC accession number 98031.

FIGS. 15A-S. Comparison of the partial and complete amino acid sequences of several plant members of the SCARECROW family of genes. The amino acid sequences are aligned in a manner that maximizes amino acid sequence similarity and identity among SCR family members. Each 25 sequence shown is continuous except where noted otherwise; the dots are inserted between two sequence segments in order to align homologous segments. "X" in the middle of a sequence indicates ambiguity in the corresponding nucleotide sequence and, possible termination of the ORF at the "X" residue site. "X" at the end of a sequence indicates termination of the ORF at the "X" residue site. The numbering of the amino acid residues is shown at the bottom

numbering of the amino acid residues is shown at the bottom of each figure and is based on the Arabidopsis SCR amino acid sequence. Conserved Motifs I through VI are indicated by the 35 various dashed lines above the figures. The new and old

names of th family members are shown in FIG. 15A. The sequenc s of SCR, Tfl and Tf4 are of the complete SCR

protein. See Table 1 for the identity and the s quence id ntifier numb r of each sequ nce shown in these figur s.

FIGS. 16A-M. The partial nucle otide sequences of several plant members of the SCARECROW family of genes. "N" 5 indicates an unknown base. See Table 1 for the identity and the sequence identifier number of each sequence shown in these figures.

FIG. 17A. The partial nucleotide sequence (SEQ ID NO:66) of the maize ZCR gene.

10 FIG. 17B. The partial amino acid sequence (SEQ ID NO:67) of the maize ZCR gene. The underlined sequence shares approximately 80% sequence identity with a corresponding sequence of Arabidopsis SCR protein.

FIG. 18. Comparison of the partial amino acid

15 sequences of several SCR ortholog sequences amplified from
the genomes of carrot, soybean and spruce. The SRPd1 and
SRPp1 sequences each were obtained by PCR amplification using
a combination of 1F and 1R primers. The SRPg1 sequence was
obtained by PCR amplification using a combination of 1F and

- 20 WP primers. The amino acid sequences are aligned in a manner that maximizes amino acid sequence identity and similarity amongst these sequences. Each sequence shown is continuous except where noted otherwise; the dashes are inserted between two sequence segments in order to allow
- 25 alignment of homologous segments. "x" in the middle of a sequence indicates ambiguity in the corresponding nucleotide sequence and, possible termination of the ORF or existence of an intron at the "x" residue site. See Table 1 for the identity and the sequence identifier number of each sequence 30 shown in this figure.

FIG. 19. Comparison of promoter activities in transgenic lines and roots. Panel a. A stably transformed line containing four copies of the B2 subdomain of the 35S promoter of CaMV upstream of GUS (Benfey et al., 1990). GUS is xpressed in the root tip. Panel b. Roots emerging from callus transformed with four copies of the B2 subdomain of

35S promoter fus d to GUS. GUS expr ssion can be se n in

the emerging root tips (arrows). Panel c. Higher magnification of a root emerging from th callus in pan 1 b. GUS is clearly restrict d t th root tip. The m rph logy of roots regenerated from calli often appears abnormal. Panel 5 d. A transgenic plant regenerated from the calli and roots shown in panel b. GUS expression in this plants appears to be similar to that of the original line shown in panel a. Panel e. ET199, a stably transformed line that contains an enhancer trapping construct with a minimal promoter fused to 10 the GUS coding region inserted 1 kb upstream from the SCR coding region. GUS expression is primarily in the endodermal layer of the root. Panel f. Roots emerging from calli transformed with the SCR promoter:: GUS construct. Expression of the GUS gene appears to be limited to an internal layer 15 (arrows). Panel g. SCR promoter:: GUS transformed root in liquid culture. Roots shown in panel f were excised and transferred to liquid cultures. GUS expression is primarily found in the endodermal layer as in ET199. The expression of GUS in the quiescent center, as seen here, is also sometimes 20 observed in ET199. Bar, $50\mu m$.

FIG. 20. Analysis of SCR promoter activity in the scr mutant background. Panel a. Roots emerging from scr calli transformed with the SCR promoter::GUS construct. Roots regenerated from scr calli are very short. GUS expression appears to be limited to an internal layer of the root (arrows). Panel b. Root regenerated from transformed scr calli and transferred to liquid culture. The scr phenotype, a single layer between the epidermis and pericycle, is easily seen. GUS expression is limited to this mutant layer. E, Epidermis. M, Mutant Layer. P, Pericycle. Bar, 50µm.

FIG. 21. Molecular Complementation of the scr mutant. Panels a, c and e. scr transformed with the SCR promoter::GUS construct. Panels b, d and f. scr transformed 35 with the SCR pr moter::SCR coding region construct. Panels a and b. Root emerging from scr calli. Arrows point to s veral very short roots among many fine root hairs in the

scr calli transformed with th SCR promoter:: GUS construct. In contrast, roots from scr calli transf rmed with th promoter::SCR coding region construct appeared to be wild-type in length, suggesting molecular complementation by 5 the transgene. Panels c and d. Transgenic roots in liquid The scr roots transformed with the SCR culture. promoter:: GUS construct appeared short, while those transformed with the SCR promoter::SCR coding region construct appeared of wild-type length. Panels e and f. 10 Transverse sections through roots emerging from calli. Whereas there is only a single cell layer between the epidermis and stele in the SCR promoter:: GUS transformed root, the radial organization of the root transformed with the SCR promoter::SCR coding region appeared identical to 15 wild-type, with both cortex and endodermal layers. E,

FIG. 22. Expression of ZCR in maize root tips.

Left Panel. Expression of ZCR is in the endodermal layer and

20 extends down through the region of the quiescent center.

Right Panel. Higher magnification showing expression in a single cell layer through the quiescent center.

epidermis. M, mutant layer. C, cortex. En, Endodermis. P,

Pericycle. Bar, 50µm

5. DETAILED DESCRIPTION OF THE INVENTION

The invention relates to the SCARECROW (SCR) gene, SCR gene products, including but not limited to transcriptional products such as mRNAs, antisense and ribozyme molecules, and translational products such as the SCR protein, polypeptides, peptides and fusion proteins related thereto; antibodies to SCR gene products; SCR regulatory regions; and the use of the foregoing to improve agronomically valuable plants.

In summary, the data described herein show the identification of SCR, a gene involved in the regulation of a specific asymmetric division, in controlling gravitropic resp ns in aerial structur s, and in controlling pattern

formation in ro ts. S quence analysis shows that the SCR protein has many hallmarks of transcription factors. In situland mark r line expression studies show that SCR is expressed in the cortex/endodermal initial of roots before asymmetric division occurs, and in quiescent center of regenerating

- division occurs, and in quiescent center of regenerating roots. Together, these findings indicate that SCR gene regulates key events that establish the asymmetric division that generates separate cortex and endodermal cell lineages, and that affect tissue organization of roots. The
- 10 establishment of these lineages is not required for cell differentiation to occur, because in the absence of division the resulting cell acquires mature characteristics of both cortex and endodermal cells. However, it is possible that SCR functions to establish the polarity of the initial before
- 15 cell division, or that it is involved in generating an external polarity that has an effect on asymmetric cell division.

Genetic analysis indicates that SCR expression affects gravitropism of plant stems and hypocotyls. This 20 indicates that SCR is also expressed in these aerial structures of plants.

The SCR genes and promoters of the present invention have a number of important agricultural uses. The SCR promoters of the invention may be used in expression 25 constructs to express desired heterologous gene products in the embryo, root, root nodule, and starch sheath layer in stem of transgenic plants transformed with such constructs. For example, SCR promoters may be used to express disease resistance genes such as lysozymes, cecropins, maganins, or 30 thionins for anti-bacterial protection or the pathogenesis-related (PR) proteins such as glucanases and chitinases for anti-fungal protection. SCR promoters also may be used to express a variety of pest resistance genes in the aforementioned plant structures and tissues. Examples of useful gene products for controlling nematodes or ins cts include Bacillus thuringiensis endotoxins, protease

inhibitors, collag nas s, chitinase, glucanases, lectins, and glycosidas s.

Gene constructs that xpr ss or ctopically express SCR, and the SCR-suppression constructs of the invention may be used to alter the root and/or stem structure, and the gravitropism of aerial structures of transgenic plants. Since SCR regulates root cell divisions, overexpression of SCR can be used to increase division of certain cells in roots and thereby form thicker and stronger roots. Thicker and stronger roots are beneficial in preventing plant lodging. Conversely, suppression of SCR expression can be used to decrease cell division in roots and thereby form thinner roots. Thinner roots are more efficient in uptake of soil nutrients. Since SCR affects gravitropism of aerial structures, overexpression of SCR may be used to develop "straighter" transgenic plants that are less susceptible to lodging.

Further, SCR gene sequence may be used as a molecular marker for a qualitative trait, e.g., a root or gravitropism trait, in molecular breeding of crop plants.

For purposes of clarity and not by way of limitation, the invention is described in the subsections below in terms of (a) SCR genes and nucleotides; (b) SCR gene products; (c) antibodies to SCR gene products; (d) SCR promoters and promoter elements; (e) transgenic plants which ectopically express SCR; (f) transgenic plants in which endogenous SCR expression is suppressed; and (g) transgenic plants in which expression of a transgene of interest is controlled by SCR promoter.

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5.1. SCR GENES

The SCARECROW genes and nucleotide sequences of the invention include: (a) a gene listed below in Table 1 (hereinafter, a gene comprising any one of the nucleotide 35 sequences shown in FIG. 5A, FIG. 8, FIG. 9, FIG. 10, FIGS. 11A-B, FIGS. 12A-B, FIGS. 16A-M, or FIG. 17A, or a segment of such nucleotide sequences), or as contained in the clones

d scribed her in and d posited with the ATCC (se Section 13, infra); (b) nucl otide sequenc that encod s a prot in comprising any one of the amin acid sequences shown in FIG. 5A, FIG. 5D, FIG. 5E, FIG. 8, FIG. 9, FIGS. 11A-B, FIGS. 13A-5 F, FIGS. 15A-S, FIG. 17B or FIG. 18 or a segment of such amino acid sequences, or that is encoded by any one of the genes and/or nucleotide sequences listed by their sequence identifier numbers in Table 1, or any segment of such genes and/or nucleotide sequences, or contained in any one of the 10 clones described herein and deposited with the ATCC (see Section 13, infra); (c) any gene comprising nucleotide sequence that hybridizes to the complement of any one of the genes and/or nucleotide sequences listed by their sequence identifier numbers in Table 1, or any segment of such genes 15 and/or nucleotide sequences, or as contained in any one of the clones described herein and deposited with the ATCC, under highly stringent conditions, e.g., hybridization to filter-bound DNA in 0.5 M NaHPO4, 7% sodium dodecyl sulfate (SDS), 1 mM EDTA at 65°C, and washing in 0.1xSSC/0.1% SDS at 20 68°C (Ausubel F.M. et al., eds., 1989, Current Protocols in Molecular Biology, Vol. I, Green Publishing Associates, Inc., and John Wiley & sons, Inc., New York, at p. 2.10.3) and that encodes a gene product functionally equivalent to SCR gene product encoded completely or partly by any one of the genes 25 and/or sequences listed in Table 1 or any segment of such genes and nucleotide sequences, or as contained in any one of the clones deposited with the ATCC; (d) any gene comprising nucleotide sequence that hybridizes to the complement of any one of the sequences listed by their sequence identifier 30 numbers in Table 1, or any segment of such nucleotide sequences, or as contained in any one of the clones described herein and deposited with the ATCC, under less stringent conditions, such as moderately stringent conditions, e.g., washing in 0.2xSSC/0.1% SDS at 42°C (Ausubel et al., 1989, 35 supra), and which encod s a functionally equivalent SCR gene

product; (e) any gene comprising nucle tide sequenc that hybridizes to the complement of any one of the sequenc s

listed by th ir sequ nce identifier numbers in Table 1 or any segment f such nucl otid sequ nc s, or as contained in any one of the clones described h rein and deposit d with the ATCC, under the following low stringency conditions: pre-5 hybridization in hybridization solution (HS) containing 43% formamide, 5xSSC, 1% SDS, 10% dextran sulfate, 0.1% sarkosyl, 2% block (Genius kit, Boehringer-Mannheim), followed by hybridization overnight at 30 to 33°C using as a probe a DNA molecule of approximately 1.6 kb of SEQ ID NO:1 at a 10 concentration of 20 ng/ml, followed by washing in 2xSSC/0.1% SDS two times for 15 minutes at room temperature and then two times at 50°C, and which encodes a functionally equivalent SCR gene product; and/or (f) any gene comprising nucleotide sequence that encodes a polypeptide or protein containing the 15 consensus sequence for SCR (i.e., MOTIF III or VHIID) shown in FIGS. 13B-D or a segment of such polypeptide or protein. The partial and complete nucleotide and amino acid sequences of SCR genes and encoded proteins and polypeptides included

in the invention are listed in Table 1 below.

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Tabl 1. SCR ORTHOLOGS AND PARALOGS

	New Name	Old Name	EST Clone ¹	SEQ ID N <u>Nucleotide' A</u>	Mos Mino Acid
5	ARABIDOPS	<u>IS</u>			
	SRPa1	1110	Z25645/33772	22	23
	SRPa2	Tf4	234599		35*
	SRPa3	3935	Z37192/1 N96166	20	21
10	SRPa4	4818	F13896/7	18	19
	SRPa5	4871	F13949	45	46
	SRPa6	12398	R29793	51	52
	SRPa7	3635	T21627 H76979 N96767	55	56
15	SRPa8	Tf1	T46205 (9468) N96653 (21711)		34*
	SRPa9	10964	T78186 T44774	47	48
	SRPa10	11261	T76483	49	50
20	SRPall	18652	N37425	53	54
	SRPa12	23196	W43803 W435138 AA042397	57	58
	SRPa13	33/08	T46008		41
25	SCR	Scr	N.A. ²	1*	2*
	RICE				
	SRP01	713	D15490		43
30	SRPo2	2504	D40482 D40607 D40800 D41389		44
	SRP03	3989	D41474		36
	SRP04	11846	C20324		59
	MAIZE				
. 35	SRPm1	18310	T18310		37
•	BRASSICA				
	SRPb1	174	H74669		42

Table 1. (Continued)

-	New Name	Old Name	EST Clone ¹	SEQ ID <u>Nucleotide'</u>	SEQ ID NOs leotide' Amino Acid	
5	SRPd1 SOYBEAN	N.A.	N.A.	60	61	
	SRPg1	N.A.	N.A.	62	63	
	SPRUCE SRPp1	N.A.	N.A.	64	65	

Each EST clone is identified by its GenBank accession number. Each EST clone corresponds to a deposit of a cDNA sequence that matches a part of the nucleotide sequence of the corresponding SCR ortholog or paralog.

N.A. = not applicable.

The partial or complete nucleotide sequence of the SCR orthologs and paralogs listed here are shown in FIGS. 5A, 8, 9, 10, 11A-B, 12A-B, 16A-M and 17A.

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- * Contains the complete coding sequence of Arabidopsis SCR gene.
- * Contains the complete amino acid sequence of Arabidopsis SRPa2, SRPa8, or SCR protein.

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Functional equival nts of th SCR gen product includ any plant gene product that regulates plant embryo or ro t d velopm nt, or, pref rably, that r gulates ro t cell division or root tissue organization, or affects gravitropism of plant aerial structures (e.g., stems and hypocotyls). Functional equivalents of the SCR gene product include naturally occurring SCR gene products, and mutant SCR gene products, whether naturally occurring or engineered.

The invention also includes nucleic acid molecules,

10 preferably DNA molecules, that hybridize to, and are
therefore the complements of the nucleotide sequences (a)
through (f), in the first paragraph of this section. Such
hybridization conditions may be highly stringent, less highly
stringent, or low stringency as described above. In

oligonucleotides ("oligos"), highly stringent conditions may refer, e.g., to washing in 6xSSC/0.05% sodium pyrophosphate at 37°C (for 14-base oligos), 48°C (for 17-base oligos), 55°C (for 20-base oligos), and 60°C (for 23-base oligos). These

20 nucleic acid molecules may act as SCR antisense molecules, useful, for example, in SCR gene regulation and/or as antisense primers in amplification reactions of SCR gene and/or nucleic acid sequences. Further, such sequences may be used as part of ribozyme and/or triple helix sequences,

25 also useful for SCR gene regulation. Still further, such molecules may be used as components in probing methods whereby the presence of a SCARECROW allele may be detected.

The invention also includes nucleic acid molecules, preferably DNA molecules, which are amplified using the 30 polymerase chain reaction under conditions described in Section 5.1.1., infra, and that encode a gene product functionally equivalent to a SCR gene product encoded by any one of the genes and sequences listed in Table 1 or as contained in any one of the clones described herein and 35 deposited with the ATCC.

The invention also encompasses (a) DNA vectors that contain any of the foregoing gene and/or coding sequences

and/or th ir compl ments (i.e., antisense or ribozyme molecules); (b) DNA xpression v ctors that contain any of th foregoing g n and/or coding sequ nces operatively associated with a regulatory element that directs the sexpression of the gene and/or coding sequences; and (c) genetically engineered host cells that contain any of the foregoing gene and/or coding sequences operatively associated with a regulatory element that directs the expression of the gene and/or coding sequences in the host cell. As used therein, regulatory elements include but are not limited to inducible and non-inducible promoters, enhancers, operators and other elements known to those skilled in the art that drive and regulate expression.

The invention also encompasses nucleotide sequences 15 that encode mutant SCR gene products, peptide fragments of the SCR gene product, truncated SCR gene products, and SCR fusion proteins. These gene products include, but are not limited to, nucleotide sequences encoding mutant SCR gene products; polypeptides or peptides corresponding to one or 20 more of the Motifs I-VI as shown in FIGS. 13A-F and FIGS. 15A-S, or the bZIP, VHIID, or leucine heptad domains of the SCR, or portions of these motifs and domains; truncated SCR gene products in which one or more of the motifs or domains is deleted, e.g., a truncated, nonfunctional SCR lacking all 25 or a portion of the Motifs I-VI as shown in FIGS. 13A-F and FIGS. 15A-S, or the bZIP, VHIID, or leucine heptad domains of the SCR. Nucleotides encoding fusion proteins may include but are not limited to full length SCR, truncated SCR or peptide fragments of SCR fused to an unrelated protein or 30 peptide, such as for example, an enzyme, fluorescent protein, or luminescent protein which can be used as a marker.

In particular, the invention includes, for example, fragments of SCR genes encoding one or more of the following

domains as shown in FIG. 5E: amino acids 1-264, 265-283, 287-316, 410-473, 436-473, and 473-653.

In addition to the general and and recoding sequences described above, homologous SCR genes, and other genes related by DNA sequence, may be identified and may be readily isolated, without undue experimentation, by molecular biological techniques well known in the art. More specifically, such homologs include, for example, paralogs (i.e., members of the SCR gene family occurring in the same plant) as well as orthologs (i.e., members of the SCR generally which occur in a different plant species) of the Arabidopsis SCR gene.

A specific embodiment of a SCR gene and coding sequence of the invention is Arabidopsis SCR (FIGS. 5A and 15 5E). Other specific embodiments include the various SCR genes and coding sequences listed in Table 1, supra.

Methods for isolating SCR genes and coding sequences are described in detail in Section 5.2, below.

SCR genes share substantial amino acid sequence
20 similarities at the protein level and nucleotide sequence
similarities in their encoding genes. The term
"substantially similar" or "substantial similarity" when used
herein with respect to two amino acid sequences means that
the two sequences have at least 75% identical residues,

- 25 preferably at least 85% identical residues and most preferably at least 95% identical residues. The same term when used herein with respect to two nucleotide sequences means that the two sequences have at least 70% identical residues, preferably at least 85% identical residues and most
- 30 preferably at least 95% identical residues. Determining whether two sequences are substantially similar may be carried out using any methodologies known to one skilled in the art, preferably using computer assisted analysis. For example, the alignments showed herein were initially
- 35 accomplished by a BLAST search (NCBI using the BLAST network server). The final alignments of SCR family members were don manually.

Moreover, SCR genes show highly localized expression in embryos and, particularly, roots. Such expressi n patterns may be asc rtain d by Northern hybridizations and in situ hybridizations using antisense 5 probes.

5.1.1. ISOLATION OF SCR GENES

The following methods can be used to obtain SCR genes and coding sequences from a wide variety of plants,

10 including but not limited to Arabidopsis thaliana, Zea mays,
Nicotiana tabacum, Daucus carota, Oryza, Glycine max, Lemna
gibba, and Picea abies.

Nucleotide sequences encoding an SCR gene or a portion thereof may be obtained by PCR amplification of plant genomic DNA or cDNA. Useful cDNA sources include "free" cDNA preparations (i.e., the products of cDNA synthesis) and cloned cDNA in cDNA libraries. Root cDNA preparations or libraries are particularly preferred.

The amplification may use, as the 5'-primer (i.e., 20 forward primer), a degenerate oligonucleotide that corresponds to a segment of a known SCR amino acid sequence, preferably from the amino-terminal region. The 3'-primer (i.e., reverse primer) may be a degenerate oligonucleotide that corresponds to a distal segment of the same known SCR 25 amino acid sequence (i.e., carboxyl to the sequence that corresponds to the 5'-primer). For example, the amino acid sequence of the Arabidopsis SCR protein (SEQ ID NO:2) may be used to design useful 5' and 3' primers. Preferably, the primers corresponds to segments in the Motif III or VHIID 30 domain of SCR protein (see FIGS. 13B-D and FIGS. 15K-L). sequence of the optimal degenerate oligonucleotide probe corresponding to a known amino acid sequence may be determined by standard algorithms known in the art. See for example, Sambrook et al., Molecular Cloning: A Laboratory

35 Manual, 2nd ed., Cold Spring Harbor Laboratory Press, Cold Spring Harbor, NY, V 1 2 (1989).

Further, for amplification from cDNA sources, the 3'-primer may be an oligonucleotid comprising an 3' oligo(dT) sequenc. The amplification may also use as primers nucleotide sequences of SCR genes or coding sequences (e.g., any one of the scr sequences and EST sequences listed in Table 1).

PCR amplification can be carried out, e.g., by use of a Perkin-Elmer Cetus thermal cycler and Taq polymerase (Gene Amp*). One can choose to synthesize several different 10 degenerate primers for use in the PCR reactions. possible to vary the stringency of hybridization conditions used in priming the PCR reactions, to allow for greater or lesser degrees of nucleotide sequence similarity between the degenerate primers and the corresponding sequences in the 15 cDNA library. One of ordinary skill in the art will know that the appropriate amplification conditions and parameters depend, in part, on the length and base composition of the primers and that such conditions may be determined using standard formulae. Protocols for executing all PCR 20 procedures discussed herein are well known to those skilled in the art, and may be found in references such as Gelfand, 1989, PCR Technology, Principles and Applications for DNA Amplification, H.A. Erlich, ed., Stockton Press, New York;

Amplification, H.A. Erlich, ed., Stockton Press, New York; and <u>Current Protocols In Molecular Biology</u>, Vol. 2, Ch. 15, 25 Ausubel et al., eds 1988, New York, Wiley & Sons, Inc.

A PCR amplified sequence may be molecularly cloned and sequenced. The amplified sequence may utilized as a probe to isolate genomic or cDNA clones of a SCR gene, as described below. This, in turn, will permit the

30 determination of a SCR gene's complete nucleotide sequence, including its promoter, the analysis of its expression, and the production of its encoded protein, as described infra.

In a preferred embodiment, PCR amplification of SCR gene and/or coding sequences can be carried out according to 35 th following proc dure:

PCT/US97/07022 WO 97/41152

PRIMERS:

Porward:

(23-mer, 2 inosines, 64-mix) SCR5AII Name:

A.A. code: **HFTANQAI**

5 DNA Sequence: 5' CAT/C TTT/C ACI GCI AAT/C CAA/G GCN AT 3'

Name: SCR5B (29-mer, 1 inosine, 144-mix)

VHIID(L/F)D A.A. code:

5' ACGTCTCGA GTI CAT/C ATA/C/T ATA/C/T GAT/C DNA Sequence:

TTN GA 3'

10 Name: 1F

A.A. code: LOCAEAV

(T/C)TI CA(A/G) TG(T/C GCI GA(A/G) GCN GT DNA Sequence:

Reverse:

(23-mer, 2 inosines, 128-mix) Name: SCR3AII

PGGPP(H/N/K)(V/L/F)R' A.A. code:

15 DNA Sequence: 5' CG/T CCA/C GTG/T TGG IGG ICC NCC NGG 3'

Name:

A.A. code: AFQVFNGI

DNA Sequence: AT ICC (A/G)TT (A/G)AA IAC (C/T)TG (A/G)AA NGC

Name:

20 A.A. code: QWPGLPHI

DNA Sequence: AT (A/G)TG (A/G)AA IA(A/G) NCC IGG CCA (C/T)TG

I = inosine N = A/C/G/T

Useful primer combinations include the following: SCR5AII+SCR3AII; SCR5B+SCR3AII; IF+IR; and IF+4R

25 PCR:

Reaction mixture (volume 50 μ 1):

-5 μ l 10X amplification buffer containing Mg (Boehringer-Mannheim)

-1 μ l 10 mM dNTP's

30 -1 μ l forward primer (stock concentration: 80 pmol/ μ l)

-1 μl reverse primer (80 pmol/μl)

-DNA (100-300 ng).

Begin reaction with "hot start" in which the enzyme is added to the mix only after a brief denaturation at a high temperature (80°C)

Cycles:

35

94°C 30 sec brief denaturation (to prevent non-specific priming) 80°C 5 min apply th enzym to the tubes (30 tubes/round maximum) 94°C 5 min thorough denaturation 2 times: 94°C 1 min 5 64°C 5 min 72°C 2 min 2 times: 94°C 1 min 62°C 5 min 72°C 2 min 2 times: 94°C 1 min 60°C 5 min 72°C 2 min 10

(reduce the annealing temperature 2°C in every second round), until 44°C is reached after that:

40 times: 94°C 20 sec 48°C 1 min 72°C 2 min

15

finally, let cool down to 15°C.

A SCR gene coding sequence may also be isolated by screening a plant genomic or cDNA library using a SCR nucleotide sequence (e.g., the sequence of any of the SCR genes and sequences and EST clone sequences listed in Table 1.) as hybridization probe. For example, the whole or a segment of the Arabidopsis SCR nucleotide sequence (FIG. 5A) may be used. Alternatively, a SCR gene may be isolated from such libraries using as probe a degenerate oligonucleotide that corresponds to a segment of a SCR amino acid sequence. For example, degenerate oligonucleotide probe corresponding to a segment of the Arabidopsis SCR amino acid sequence (FIG. 5E) may be used.

In preparation of cDNA libraries, total RNA is isolated from plant tissues, preferably roots. Poly(A)+ RNA is isolated from the total RNA, and cDNA prepared from the poly(A)+ RNA, all using standard procedures. See, for example, Sambrook et al., Molecular Cloning: A Laboratory

Manual, 2d ed., Vol. 2 (1989). The cDNAs may be synthesized with a r striction enzyme site at their 3'-ends by using an appropriate primer and further have linkers or adaptors

attached at their 5'-ends to facilitate the insertion of the cDNAs into suitable cDNA cloning vectors. Alternatively, adaptors or link rs may be attached to the cDNAs after the completion of cDNA synthesis.

isolated and fragments are generated, some of which will encode parts of the whole SCR protein. The DNA may be cleaved at specific sites using various restriction enzymes. Alternatively, one may use DNase in the presence of manganese to fragment the DNA, or the DNA can be physically sheared, as for example, by sonication. The DNA fragments can then be separated according to size by standard techniques, including but not limited to, agarose and polyacrylamide gel electrophoresis, column chromatography and sucrose gradient to centrifugation.

The genomic DNA or cDNA fragments can be inserted into suitable vectors, including but not limited to, plasmids, cosmids, bacteriophages lambda or T4, and yeast artificial chromosome (YAC) [See, for example, Sambrook et al., Molecular Cloning: A Laboratory Manual, 2d ed., Cold Spring Harbor Laboratory Press, Cold Spring Harbor, New York (1989); Glover, D.M(ed.), DNA Cloning: A Practical Approach,

MRL Press, Ltd., Oxford, U.K., Vols. I and II (1985)].

The SCR nucleotide probe, DNA or RNA, should be at

25 least 17 nucleotides, preferably at least 26 nucleotides, and
most preferably at least 50 nucleotides in length. The
nucleotide probe is hybridized under moderate stringency
conditions and washed under moderate, preferably high
stringency conditions. Clones in libraries with insert DNA

30 having substantial homology to the SCR probe will hybridize
to the probe. Hybridization of the nucleotide probe to
genomic or cDNA libraries is carried out using methods known
in the art. One of ordinary skill in the art will know that
the appropriate hybridization and wash conditions depend on

35 the length and base composition of the probe and that such
conditions may be determin d using standard formulae. Se,
for xampl, Sambrook t al., Molecular Cloning: A Laboratory

Manual, 2nd d., Cold Spring Harbor Laboratory Press, Cold Spring Harbor, NY, Vol. 2, (1989) pp 11.45-11.57 and 15.55-15.57.

The identity of a cloned or amplified SCR gene

5 sequence can be verified by comparing the amino acid
sequences of its three open reading frames with the amino
acid sequence of a SCR gene (e.g., Arabidopsis SCR protein
[SEQ ID No:2]). A SCR gene or coding sequence encodes a
protein or polypeptide whose amino acid sequence is

10 substantially similar to that of a SCR protein or polypeptide
(e.g., the amino acid sequence of any one of the SCR proteins
and/or polypeptides shown in FIG. 5A, 5E, FIG. 8, FIG. 9,
FIGS. 11A-B, FIGS. 15A-S, FIG. 17B and FIG. 18). The
identity of the cloned or amplified SCR gene sequence may be
15 further verified by examining its expression pattern, which
should show highly localized expression in the embryo and/or
root of the plant from which the SCR gene sequence was
isolated.

Comparison of the amino acid sequences encoded by a 20 cloned or amplified sequence may reveal that it does not contain the entire SCR gene or its promoter. In such a case the cloned or amplified SCR gene sequence may be used as a probe to screen a genomic library for clones having inserts that overlap the cloned or amplified SCR gene sequence. A 25 complete SCR gene and its promoter may be reconstructed by splicing the overlapping SCR gene sequences.

5.1.2. EXPRESSION OF SCR GENE PRODUCTS

SCR proteins, polypeptides and peptide fragments,

30 mutated, truncated or deleted forms of SCR and/or SCR fusion
proteins can be prepared for a variety of uses, including but
not limited to the generation of antibodies, as reagents in
assays, the identification of other cellular gene products
involved in regulation of root development; etc.

SCR translational products includ, but are not limited t those pr teins and polypeptides encoded by th SCR gene sequ nces described in Section 5.1, above. The

invention enc mpass s prot ins that are functionally equivalent to the SCR gene products described in Section 5.1. Such a SCR gene product may contain one r more deletions, additions or substitutions of SCR amino acid residues within 5 the amino acid sequence encoded by any one of the SCR gene sequences described, above, in Section 5.1, but which result in a silent change, thus producing a functionally equivalent SCR gene product. Amino acid substitutions may be made on the basis of similarity in polarity, charge, solubility, hydrophobicity, hydrophilicity, and/or the amphipathic nature of the residues involved.

For example, nonpolar (hydrophobic) amino acids include alanine, leucine, isoleucine, valine, proline, phenylalanine, tryptophan, and methionine; polar neutral 15 amino acids include glycine, serine, threonine, cysteine, tyrosine, asparagine, and glutamine; positively charged (basic) amino acids include arginine, lysine, and histidine; and negatively charged (acidic) amino acids include aspartic acid and glutamic acid. "Functionally equivalent", as 20 utilized herein, refers to a protein capable of exhibiting a substantially similar in vivo activity as the endogenous SCR gene products encoded by the SCR gene sequences described in Section 5.1, above. Alternatively, "functionally equivalent" may refer to peptides capable of regulating gene expression 25 in a manner substantially similar to the way in which the corresponding portion of the endogenous SCR gene product would.

and polypeptides that agree not functionally equivalent to

30 the gene products described in Section 5.1. Such a mutant

SCR protein or polypeptide may contain one or more deletions,

additions or substitutions of SCR amino acid residues within

the amino acid sequence encoded by any one the SCR gene

sequences described above in Section 5.1., and which result

35 in loss of on or m r functions of the SCR protein (e.g.,

recognition of a specific nucl ic sequence, binding of an

transcription fact r, etc.), thus producing a SCR gene

product not functionally equivalent to the wild-typ SCR protein.

While random mutations can be made to SCR DNA (using random mutagenesis techniques well known to those 5 skilled in the art) and the resulting mutant SCRs tested for activity, site-directed mutations of the SCR gene and/or coding sequence can be engineered (using site-directed mutagenesis techniques well known to those skilled in the art) to generate mutant SCRs with increased function, (e.g.,

- 10 resulting in improved root formation), or decreased function (e.g., resulting in suboptimal root function). In particular, mutated SCR proteins in which any of the domains shown in FIGS. 13A-F are deleted or mutated are within the scope of the invention. Additionally, peptides corresponding
- 15 to one or more domains of the SCR (e.g., shown in FIGS. 13A-F), truncated or deleted SCRs, as well as fusion proteins in which the full length SCR, a SCR polypeptide or peptide fused to an unrelated protein are also within the scope of the invention and can be designed on the basis of the SCR
- 20 nucleotide and SCR amino acid sequences disclosed in Section 5.1. above.

While the SCR polypeptides and peptides can be chemically synthesized (e.g., see Creighton, 1983, Proteins: Structures and Molecular Principles, W.H. Freeman & Co.,

25 N.Y.) large polypeptides derived from SCR and the full length SCR may advantageously be produced by recombinant DNA technology using techniques well known to those skilled in the art for expressing nucleic acid sequences.

Methods which are well known to those skilled in 30 the art can be used to construct expression vectors containing SCR protein coding sequences and appropriate transcriptional/translational control signals. These methods include, for example, in vitro recombinant DNA techniques, synthetic techniques and in vivo recombination/genetic

35 recombination. See, for example, the techniques described in Sambrook et al., 1989, supra, and Ausubel t al., 1989, supra. Alternatively, RNA capable of enc ding SCR protein

sequences may be chemically synthesized using, for example, synthesiz rs. See, for exampl, the techniques d scribed in "Oligonucleotide Synthesis", 1984, Gait, M.J. ed., IRL Press, Oxford.

A variety of host-expression vector systems may be utilized to express the SCR gene products of the invention. Such host-expression systems represent vehicles by which the SCR gene products of interest may be produced and subsequently recovered and/or purified from the culture or 10 plant (using purification methods well known to those skilled in the art), but also represent cells which may, when transformed or transfected with the appropriate nucleotide coding sequences, exhibit the SCR protein of the invention in These include but are not limited to microorganisms 15 such as bacteria (e.g., E. coli, B. subtilis) transformed with recombinant bacteriophage DNA, plasmid DNA or cosmid DNA expression vectors containing SCR protein coding sequences; yeast (e.g., Saccharomyces, Pichia) transformed with recombinant yeast expression vectors containing the SCR 20 protein coding sequences; insect cell systems infected with recombinant virus expression vectors (e.g., baculovirus) containing the SCR protein coding sequences; plant cell systems infected with recombinant virus expression vectors (e.g., cauliflower mosaic virus, CaMV; tobacco mosaic virus, 25 TMV) or transformed with recombinant plasmid expression vectors (e.g., Ti plasmid) containing SCR protein coding sequences; or mammalian cell systems (e.g., COS, CHO, BHK, 293, 3T3) harboring recombinant expression constructs containing promoters derived from the genome of mammalian 30 cells (e.g., metallothionein promoter) or from mammalian

In bacterial systems, a number of expression

35 vectors may be advantageously selected depending upon the use intended for the SCR protein being expressed. For example, when a large quantity of such a protein is to be produc d,

viruses (e.g., the adenovirus late promoter; the vaccinia virus 7.5K promoter; the cytomegalovirus promoter/enhancer;

etc.).

for the gen ration f antibodies or to screen peptid libraries, for example, v ctors which direct the expression of high levels of fusion protein products that are readily purified may be desirable. Such vectors include, but are not limited, to the E. coli expression vector pUR278 (Ruther et al., 1983, EMBO J. 2:1791), in which the SCR coding sequence may be ligated individually into the vector in frame with the lac Z coding region so that a fusion protein is produced; pIN vectors (Inouye & Inouye, 1985, Nucleic Acids Res. 13:3101-

- 10 3109; Van Heeke & Schuster, 1989, J. Biol. Chem. 264:5503-5509); and the like. pGEX vectors may also be used to express foreign polypeptides as fusion proteins with glutathione S-transferase (GST). In general, such fusion proteins are soluble and can easily be purified from lysed cells by
- 15 adsorption to glutathione-agarose beads followed by elution in the presence of free glutathione. The pGEX vectors are designed to include thrombin or factor Xa protease cleavage sites so that the cloned target gene protein can be released from the GST moiety.
- In one such embodiment of a bacterial system, full length cDNA sequences are appended with in-frame Bam HI sites at the amino terminus and Eco RI sites at the carboxyl terminus using standard PCR methodologies (Innis et al., 1990, supra) and ligated into the pGEX-2TK vector (Pharmacia,
- 25 Uppsala, Sweden). The resulting cDNA construct contains a kinase recognition site at the amino terminus for radioactive labelling and glutathione S-transferase sequences at the carboxyl terminus for affinity purification (Nilsson, et al., 1985, EMBO J. 4: 1075; Zabeau and Stanley, 1982, EMBO J. 1:

The recombinant constructs of the present invention may include a selectable marker for propagation of the construct. For example, a construct to be propagated in bacteria preferably contains an antibiotic resistance gene, such as one that conf rs resistance to kanamycin, tetracyclin, strept mycin, or chloramphenicol. Suitable

vectors for propagating the c nstruct include plasmids, cosmids, bact riophages or virus s, to name but a few.

In addition, the rec mbinant constructs may include plant-expressible, selectable, or screenable marker genes for 5 isolating, identifying or tracking plant cells transformed by these constructs. Selectable markers include, but are not limited to, genes that confer antibiotic resistance, (e.g., resistance to kanamycin or hygromycin) or herbicide resistance (e.g., resistance to sulfonylurea,

10 phosphinothricin, or glyphosate). Screenable markers include, but are not be limited to, genes encoding 8-glucuronidase (Jefferson, 1987, Plant Mol. Biol. Rep. 5:387-405), luciferase (Ow et al., 1986, Science 234:856-859), B protein that regulates anthocyanin pigment production (Goff 15 et al., 1990, EMBO J 9:2517-2522).

In embodiments of the present invention which utilize the Agrobacterium tumefacien system for transforming plants (see infra), the recombinant constructs may additionally comprise at least the right T-DNA border sequences flanking the DNA sequences to be transformed into the plant cell. Alternatively, the recombinant constructs may comprise the right and left T-DNA border sequences flanking the DNA sequence. The proper design and construction of such T-DNA based transformation vectors are well known to those skilled in the art.

5.1.3. ANTIBODIES TO SCR PROTEINS AND POLYPEPTIDES
Antibodies that specifically recognize one or more
epitopes of SCR, or epitopes of conserved variants of SCR, or
30 peptide fragments of the SCR are also encompassed by the
invention. Such antibodies include but are not limited to
polyclonal antibodies, monoclonal antibodies (mAbs),
humanized or chimeric antibodies, single chain antibodies,
Fab fragments, F(ab'), fragments, fragments produced by a Fab
35 expression library, anti-idiotypic (anti-Id) antibodies, and
epitope-binding fragments of any f the above.

For the production f antibodies, various host animals may be immunized by inj ction with th SCR protein, an SCR peptide (e.g., one corresponding to a functional domain of the protein), a truncated SCR polypeptide (SCR in 5 which one or more domains has been deleted), functional equivalents of the SCR protein, or mutants of the SCR protein. Such SCR proteins, polypeptides, peptides or fusion proteins can be prepared and obtained as described in Section 5.1.2. supra. Host animals may include but are not limited 10 to rabbits, mice, and rats, to name but a few. Various adjuvants may be used to increase the immunological response, depending on the host species, including but not limited to Freund's (complete and incomplete), mineral gels such as aluminum hydroxide, surface active substances such as 15 lysolecithin, pluronic polyols, polyanions, peptides, oil emulsions, keyhole limpet hemocyanin, dinitrophenol, and potentially useful human adjuvants such as BCG (bacille Calmette-Guerin) and Corynebacterium parvum. Polyclonal antibodies are heterogeneous populations of antibody 20 molecules derived from the sera of the immunized animals. Monoclonal antibodies, which are homogeneous populations of antibodies to a particular antigen, may be obtained by any technique which provides for the production of antibody molecules by continuous cell lines in culture. 25 These include, but are not limited to, the hybridoma technique of Kohler and Milstein, (Nature 256:495-497 [1975]; and U.S. Patent No. 4,376,110), the human B-cell hybridoma technique (Kosbor et al., 1983, Immunology Today 4:72; Cole et al., 1983, Proc. Natl. Acad. Sci. USA 80:2026-2030), and 30 the EBV-hybridoma technique (Cole et al., 1985, Monoclonal Antibodies And Cancer Therapy, Alan R. Liss, Inc., pp. 77-96). Such antibodies may be of any immunoglobulin class including IgG, IgM, IgE, IgA, IgD and any subclass thereof. The hybridoma producing the mAb of this invention may be 35 cultivated in vitro or in vivo. Production of high titers of mabs in vivo mak s this th presently pr ferred method of

pr duction.

In addition, t chniques developed for the production of "chimeric antibodies" (Morris net al., 1984, Proc. Natl. Acad. Sci., 81:6851-6855; Neuberger et al., 1984, Nature, 312:604-608; Takeda et al., 1985, Nature, 314:452-5454) by splicing the genes from a mouse antibody molecule of appropriate antigen specificity together with genes from a human antibody molecule of appropriate biological activity can be used. A chimeric antibody is a molecule in which different portions are derived from different animal species, such as those having a variable region derived from a murine mab and a human immunoglobulin constant region.

In addition, techniques have been developed for the production of humanized antibodies. (See, e.g., Queen, U.S. Patent No. 5,585,089.) An immunoglobulin light or heavy

15 chain variable region consists of a "framework" region interrupted by three hypervariable regions, referred to as complementarily determining regions (CDRs). The extent of the framework region and CDRs have been precisely defined (see, "Sequences of Proteins of Immunological Interest",

20 Kabat, E. et al., U.S. Department of Health and Human Services (1983). Briefly, humanized antibodies are antibody

molecules from non-human species having one or more CDRs from

the non-human species and a framework region from a human

Alternatively, techniques described for the production of single chain antibodies (U.S. Patent 4,946,778; Bird, 1988, Science 242:423-426; Huston et al., 1988, Proc. Natl. Acad. Sci. USA 85:5879-5883; and Ward et al., 1989, Nature 334:544-546) can be adapted to produce single chain antibodies against SCR proteins or polypeptides. Single chain antibodies are formed by linking the heavy and light chain fragments of the Fv region via an amino acid bridge, resulting in a single chain polypeptide.

Antibody fragments which recognize specific

35 epitop s may be g nerated by known t chniques. For example, such fragments includ but are not limited to: the F(ab')₂ fragments which can be produced by pepsin dig stion of the

antibody molecul and the Fab fragments which can be generat d by r ducing the disulfide bridges of the F(ab')₂ fragments. Alternatively, Fab expr ssion libraries may be constructed (Huse et al., 1989, Science, 246:1275-1281) to allow rapid and easy identification of monoclonal Fab fragments with the desired specificity.

Antibodies to a SCR protein and/or polypeptide can, in turn, be utilized to generate anti-idiotype antibodies that "mimic" SCR, using techniques well known to those

10 skilled in the art. (See, e.g., Greenspan & Bona, 1993, FASEB J 7(5):437-444; and Nissinoff, 1991, J. Immunol. 147(8):2429-2438).

5.1.4. SCR GENE OR GENE PRODUCTS AS MARKERS FOR QUALITATIVE TRAIT LOCI

Any of the nucleotide sequences (including EST clone sequences) described in \$\$ 5.1 and 5.1.1. and/or listed in Table 1, and/or polypeptides and proteins described in \$\$ 5.1.2. and/or listed in Table 1, can be used as markers for qualitative trait loci in breeding programs for crop plants. To this end, the nucleic acid molecules, including but not limited to full length SCR coding sequences, and/or partial sequences (ESTs), can be used in hybridization and/or DNA amplification assays to identify the endogenous SCR genes, scr mutant alleles and/or SCR expression products in cultivars as compared to wild-type plants. They can also be used as markers for linkage analysis of qualitative trait loci. It is also possible that the SCR gene may encode a product responsible for a qualitative trait that is desirable

30 in a crop breeding program. Alternatively, the SCR protein, peptides and/or antibodies can be used as reagents in immunoassays to detect expression of the SCR gene in cultivars and wild-type plants.

15

5.2. SCR PROMOTERS

described herein.

According t the pr s nt inv ntion, SCR pr moters and functional p rti ns th r of d scribed h rein r f r to regions of the SCR gene which are capable of promoting 5 tissue-specific expression in embryos and/or roots of an operably linked coding sequence in plants. The SCR promoter described herein refers to the regulatory elements of SCR genes, i.e., regulatory regions of genes which are capable of selectively hybridizing to the nucleic acids described in 10 Section 5.1, or regulatory sequences contained, for example, in the region between the translational start site of the Arabidopsis SCR gene and the HindIII site approximately 2.5 kb upstream of the site in plasmid pLIG1-3/SAC+Mob21SAC (see FIGS. 5A and 14) in hybridization assays, or which are 15 homologous by sequence analysis (containing a span of 10 or more nucleotides in which at least 50 percent of the nucleotides are identical to the sequences presented herein). Homologous nucleotide sequences refer to nucleotide sequences including, but not limited to, SCR promoters in diverse plant 20 species (e.g., promoters of orthologs of Arabidopsis SCR) as well as genetically engineered derivatives of the promoters

Methods which could be used for the synthesis, isolation, molecular cloning, characterization and 25 manipulation of SCR promoter sequences are well known to those skilled in the art. See, e.g., the techniques described in Sambrook et al., Molecular Cloning: A Laboratory Manual, 2nd. ed., Cold Spring Harbor Laboratory, Cold Spring Harbor, New York (1989).

According to the present invention, SCR promoter sequences or portions thereof described herein may be obtained from appropriate plant or mammalian sources from cell lines or recombinant DNA constructs containing SCR promoter sequences, and/or by chemical synthetic methods.

35 SCR promoter sequences can be obtained from genomic clones centaining sequences 5' upstream of SCR coding sequences.

Such 5' upstream clones may be obtained by screening genomic

libraries using SCR protein coding sequences, particularly those incoding SCR N-terminal sequences, from SCR gen clones obtained as described in Secti ns 5.1. and 5.2. Standard methods that may used in such screening include, for example, 5 the method set forth in Benton & Davis, 1977, Science 196:180 for bacteriophage libraries; and Grunstein & Hogness, 1975, Proc. Nat. Acad. Sci. U.S.A. 72:3961-3965 for plasmid libraries.

The full extent and location of SCR promoters

10 within such 5' upstream clones may be determined by the functional assay described below. In the event a 5' upstream clone does not contain the entire SCR promoter as determined by the functional assay, the insert DNA of the clone may be used to isolate genomic clones containing sequences further

- 15 5' upstream of the SCR coding sequences. Such further upstream sequences can be spliced on to existing 5' upstream sequences and the reconstructed 5' upstream region tested for functionality as a SCR promoter (i.e., promoting tissuespecific expression in embryos and/or roots of an operably
- 20 linked gene in plants). This process may be repeat until the complete SCR promoter is obtained.

The location of the SCR promoter within genomic sequences 5' upstream of the SCR gene isolated as described above may be determined using any method known in the art.

- 25 For example, the 3'-end of the promoter may be identified by locating the transcription initiation site, which may be determined by methods such as RNase protection (e.g., Liang et al., 1989, J. Biol. Chem. 264:14486-14498), primer extension (e.g., Weissenborn & Larson, 1992, J. Biol. Chem.
- 30 267:6122-6131), and/or reverse transcriptase/PCR. The location of the 3'-end of the promoter may be confirmed by sequencing and computer analysis, examining for the canonical AGGA or TATA boxes of promoters that are typically 50-60 base pairs (bp) and 25-35 bp 5'-upstream of the transcription
- 35 initiation site. Th 5'- nd promoter may be d fined by del ting s quences fr m the 5'- nd f the promoter containing fragm nt, c nstructing a transcriptional or translational

fusion of the r sected fragment and a report r g n , and xamining the xpression characteristics of th chim ric g ne in transgenic plants. R port r gen s that may be used to such ends include, but are not limited to, GUS, CAT,
5 luciferase, β-galactosidase and C1 and R gene controlling anthocyanin production.

According to the present invention, a SCR promoter is one that confers to an operably linked gene in a transgenic plant tissue-specific expression in roots, root nodules, stems and/or embryos. A SCR promoter comprises the region between about -5,000 bp and +1 bp upstream of the transcription initiation site of SCR gene. In a particular embodiment, the Arabidopsis SCR promoter comprises the region between positions -2.5 kb and +1 in the 5' upstream region of the Arabidopsis SCR gene (see FIGS. 5A and 14).

5.2.1. CIS-REGULATORY ELEMENTS OF SCR PROMOTERS
According to the present invention, the cisregulatory elements within a SCR promoter may be identified
20 using any method known in the art. For example, the location
of cis-regulatory elements within an inducible promoter may
be identified using methods such as DNase or chemical
footprinting (e.g., Meier et al., 1991, Plant Cell 3:309-315)
or gel retardation (e.g., Weissenborn & Larson, 1992, J.
25 Biol. Chem. 267-6122-6131; Beato, 1989, Cell 56:335-344;
Johnson et al., 1989, Ann. Rev. Biochem. 58:799-839).
Additionally, resectioning experiments may also be employed
to define the location of the cis-regulatory elements. For
example, an inducible promoter-containing fragment may be

30 resected from either the 5' or 3'-end using restriction enzyme or exonuclease digests.

To determine the location of cis-regulatory elements within the sequence containing the inducible

promoter, the 5'- or 3'-resected fragments, internal
35 fragments to the inducible promoter containing sequence, or
inducible promoter fragments containing s quences identified
by fo tprinting or gel retardation exp riments may be fused

to th 5'-end of a truncated plant promoter, and the activity of the chim ric promoter in transg nic plant examin d. Useful truncat d prom ters to thes ends comprise sequences starting at or about the transcription initiation site and 5 extending to no more than 150 bp 5' upstream. truncated promoters generally are inactive or are only minimally active. Examples of such truncated plant promoters may include, among others, a "minimal" CaMV 35S promoter whose 5' end terminates at position -46 bp with respect to 10 the transcription initiation site (Skriver et al., Proc. Natl. Acad. Sci. USA 88:7266-7270); the truncated "-90 355" promoter in the X-GUS-90 vector (Benfey & Chua, 1989, Science 244:174-181); a truncated "-101 nos" promoter derived from the nopaline synthase promoter (Aryan et al., 1991, Mol. Gen. 15 Genet. 225:65-71); and the truncated maize Adh-1 promoter in pADcat 2 (Ellis et al., 1987, EMBO J. 6:11-16).

According to the present invention, a cisregulatory element of a SCR promoter is a sequence that
confers to a truncated promoter tissue-specific expression in
20 embryos, stems, root nodules and/or roots.

5.2.2. SCR PROMOTER-DRIVEN EXPRESSION VECTORS
The properties of the nucleic acid sequences are
varied as are the genetic structures of various potential
25 host plant cells. In the preferred embodiments of the
present invention, described herein, a number of features
which an artisan may recognize as not being absolutely
essential, but clearly advantageous are used. These include
methods of isolation, synthesis or construction of gene
30 constructs, the manipulation of the gene constructs to be
introduced into plant cells, certain features of the gene
constructs, and certain features of the vectors associated
with the gene constructs.

Further, the gene constructs of the present

35 invention may be encoded on DNA or RNA molecul s. According to the pr s nt inv nti n, it is preferred that the d sired, stable genotypic change of the target plant b effected

through genomic integration of xogenously introduc d nucleic acid construct(s), particularly recombinant DNA constructs.

N nethel ss, according to the present invention, such genotypic changes can also be effected by the introduction of 5 episomes (DNA or RNA) that can replicate autonomously and that are somatically and germinally stable. Where the introduced nucleic acid constructs comprise RNA, plant transformation or gene expression from such constructs may proceed through a DNA intermediate produced by reverse 10 transcription.

The present invention provides for use of recombinant DNA constructs which contain tissue-specific and developmental-specific promoter fragments and functional portions thereof. As used herein, a functional portion of a 15 SCR promoter is capable of functioning as a tissue-specific promoter in the embryo, stem, root nodule and/or root of a plant. The functionality of such sequences can be readily established by any method known in the art. Such methods include, for example, constructing expression vectors with such sequences and determining whether they confer tissue-specific expression in the embryo, stem, root nodule and/or root to an operably linked gene. In a particular embodiment, the invention provides for the use of the Arabidopsis SCR promoter contained in the sequences depicted in FIGS. 5A and 25 14 and the insert DNA of plasmid pGEX-2TK*.

The SCR promoters of the invention may be used to direct the expression of any desired protein, or to direct the expression of a RNA product, including, but not limited to, an "antisense" RNA or ribozyme. Such recombinant 30 constructs generally comprise a native SCR promoter or a recombinant SCR promoter derived therefrom, ligated to the nucleic acid sequence encoding a desired heterologous gene product.

A recombinant SCR promoter is used herein to refer

35 to a promoter that c mprises a functional portion of a native

SCR promoter or a promoter that contains nativ pr moter

sequenc s that is modified by a regulatory element from a SCR

promot r. Alternatively, a recombinant inducible promot r d riv d from the scr promoter may be a chimeric promoter, comprising a full-length or truncat d plant prom ter modified by the attachment of one or more SCR cis-regulatory elements.

- The manner of chimeric promoter constructions may be any well known in the art. For examples of approaches that can be used in such constructions, see Section 5.1.2., above and Fluhr et al., 1986, Science 232:1106-1112; Ellis et al., 1987, EMBO J. 6:11-16; Strittmatter & Chua, 1987, Proc.
- 10 Natl. Acad. Sci. USA 84:8986-8990; Poulsen & Chua, 1988, Mol. Gen. Genet. 214:16-23; Comai et al., 1991, Plant Mol. Biol. 15:373-381; Aryan et al., 1991, Mol. Gen. Genet. 225:65-71.

According to the present invention, where a SCR promoter or a recombinant SCR promoter is used to express a 15 desired protein, the DNA construct is designed so that the protein coding sequence is ligated in phase with the translational initiation codon downstream of the promoter. Where the promoter fragment is missing 5'leader sequences, a DNA fragment encoding both the protein and its 5' RNA leader

- 20 sequence is ligated immediately downstream of the transcription initiation site. Alternatively, an unrelated 5' RNA leader sequence may be used to bridge the promoter and the protein coding sequence. In such instances, the design should be such that the protein coding sequence is ligated in
- 25 phase with the initiation codon present in the leader sequence, or ligated such that no initiation codon is interposed between the transcription initiation site and the first methionine codon of the protein.

Further, it may be desirable to include additional 30 DNA sequences in the protein expression constructs. Examples of additional DNA sequences include, but are not limited to, those encoding: a 3' untranslated region; a transcription termination and polyadenylation signal; an intron; a signal peptide (which facilitates the secretion of the protein); or 35 a transit peptide (which targets the prot in to a particular cellular compartment such as the nucleus, chloroplast, mitochondria, or vacuole).

5.3. PRODUCTION OF TRANSGENIC PLANTS AND PLANT CELLS

plant or plant cell may be obtained by transforming a plant cell with the nucleic acid constructs described herein. In some instances, it may be desirable to engineer a plant or plant cell with several different gene constructs. Such engineering may be accomplished by transforming a plant or plant cell with all of the desired gene constructs simultaneously. Alternatively, the engineering may be carried out sequentially. That is, transforming with one gene construct, obtaining the desired transformant after selection and screening, transforming the transformant with a second gene construct, and so on.

In an embodiment of the present invention, 15 Agrobacterium is employed to introduce the gene constructs Such transformations preferably use binary into plants. Agrobacterium T-DNA vectors (Bevan, 1984, Nuc. Acid Res. 12:8711-8721), and the co-cultivation procedure (Horsch et al., 1985, Science 227:1229-1231). Generally, the Agrobacterium transformation system is used to engineer dicotyledonous plants (Bevan et al., 1982, Ann. Rev. Genet. 16:357-384; Rogers et al., 1986, Methods Enzymol. 118:627-641). The Agrobacterium transformation system may also be used to transform, as well as transfer, DNA to monocotyledonous plants and plant cells (see Hernalsteen et al., 1984, EMBO J 3:3039-3041; Hooykass-Van Slogteren et al., 1984, Nature 311:763-764; Grimsley et al., 1987, Nature 325:1677-179; Boulton et al., 1989, Plant Mol. Biol. 12:31-30 40.; Gould et al., 1991, Plant Physiol. 95:426-434).

In other embodiments, various alternative methods for introducing recombinant nucleic acid constructs into plants and plant cells may also be utilized. These other methods are particularly useful where the target is a monocotyledonous plant or plant cell. Alternative gene transf r and transformati n methods include, but are not limited to, protoplast transformation thr ugh calcium-,

polyethylene glycol (PEG) - or el ctroporation-mediated uptake of nak d DNA (see Paszkowski et al., 1984, EMBO J 3:2717-2722, Potrykus et al., 1985, Mol. Gen. Genet. 199:169-177; Fromm et al., 1985, Proc. Natl. Acad. Sci. USA 82:5824-5828; Shimamoto, 1989, Nature 338:274-276), and electroporation of plant tissues (D'Halluin et al., 1992, Plant Cell 4:1495-1505). Additional methods for plant cell transformation include microinjection, silicon carbide mediated DNA uptake (Kaeppler et al., 1990, Plant Cell Reporter 9:415-418), and microprojectile bombardment (see Klein et al., 1988, Proc. Natl. Acad. Sci. USA 85:4305-4309; Gordon-Kamm et al., 1990, Plant Cell 2:603-618).

According to the present invention, a wide variety of plants may be engineered for the desired physiological and 15 agronomic characteristics described herein using the nucleic acid constructs of the instant invention and the various transformation methods mentioned above. In preferred embodiments, target plants for engineering include, but are not limited to, crop plants such as maize, wheat, rice, 20 soybean, tomato, tobacco, carrots, peanut, potato, sugar beets, sunflower, yam, Arabidopsis, rape seed, and petunia; and trees such as spruce.

According to the present invention, desired plants and plant cells may be obtained by engineering the gene

25 constructs described herein into a variety of plant cell types, including but not limited to, protoplasts, tissue culture cells, tissue and organ explants, pollen, embryos as well as whole plants. In an embodiment of the present invention, the engineered plant material is selected or

30 screened for transformants (i.e., those that have incorporated or integrated the introduced gene construct(s)) following the approaches and methods described below. An isolated transformant may then be regenerated into a plant. Alternatively, the engineered plant material may be

35 regenerated into a plant, or plantl t, b f re subjecting th deriv d plant, r plantlet, t s l ction or scre ning for the marker gen traits. Procedures for r generating plants from

plant cells, tissus or organs, ither before or after selecting or screening for mark r gen (s), are well known to those skilled in the art.

A transformed plant cell, callus, tissue or plant
may be identified and isolated by selecting or screening the
engineered plant material for traits encoded by the marker
genes present on the transforming DNA. For instance,
selection may be performed by growing the engineered plant
material on media containing inhibitory amounts of the
antibiotic or herbicide to which the transforming marker gene
construct confers resistance. Further, transformed plants
and plant cells may also be identified by screening for the
activities of any visible marker genes (e.g., the ßglucuronidase, luciferase, B or C1 genes) that may be present
on the recombinant nucleic acid constructs of the present
invention. Such selection and screening methodologies are
well known to those skilled in the art.

Physical and biochemical methods may also be used to identify a plant or plant cell transformant containing the 20 gene constructs of the present invention. These methods include but are not limited to: 1) Southern analysis or PCR amplification for detecting and determining the structure of the recombinant DNA insert; 2) Northern blot, S-1 RNase protection, primer-extension or reverse transcriptase-PCR 25 amplification for detecting and examining RNA transcripts of the gene constructs; 3) enzymatic assays for detecting enzyme or ribozyme activity, where such gene products are encoded by the gene construct; 4) protein gel electrophoresis, western blot techniques, immunoprecipitation, or enzyme-linked 30 immunoassays, where the gene construct products are proteins; 5) biochemical measurements of compounds produced as a consequence of the expression of the introduced gene constructs. Additional techniques, such as in situ hybridization, enzyme staining, and immunostaining, may also 35 be used to detect the presence or expression of the recombinant construct in sp cific plant organs and tissues.

The m thods for doing all these assays are w ll known to those skilled in th art.

5

5.3.1. TRANSGENIC PLANTS THAT ECTOPICALLY EXPRESS SCR

In accordance to the present invention, a plant that expresses a recombinant SCR gene may be engineered by transforming a plant cell with a gene construct comprising a plant promoter operably associated with a sequence encoding SCR protein or a fragment thereof. (Operably associated is used herein to mean that transcription controlled by the "associated" promoter would produce a functional messenger RNA, whose translation would produce the enzyme.) promoter may be constitutive or inducible. Useful 15 constitutive promoters include, but are not limited to, the CaMV 35S promoter, the T-DNA mannopine synthetase promoter, and their various derivatives. Useful inducible promoters include but are not limited to the promoters of ribulose bisphosphate carboxylase (RUBISCO) genes, chlorophyll a/b 20 binding protein (CAB) genes, heat shock genes, the defense responsive gene (e.g., phenylalanine ammonia lyase genes), wound induced genes (e.g., hydroxyproline rich cell wall protein genes), chemically-inducible genes (e.g., nitrate reductase genes, gluconase genes, chitinase genes, PR-1 genes etc.), dark-inducible genes (e.g., asparagine synthetase gene (Coruzzi and Tsai, U.S. Patent 5,256,558, October 26, 1993, Gene Encoding Plant Asparagine Synthetase) developmentally regulated genes (e.g., Shoot Meristemless gene) to name just a few.

In yet another embodiment of the present invention, it may be advantageous to transform a plant with a gene construct operably linking a modified or artificial promoter to a sequence encoding SCR protein or a fragment thereof.

Typically, such promoters, constructed by recombining structural elements of different promoters, have unique xpr ssi n patterns and/or levels n t found in natural promot rs. Se, e.g., Salina et al., 1992, Plant Cell

4:1485-1493, for examples f artificial pr mot rs constructed from combining cis-regulat ry lements with a promot r core.

In a pr ferred mbodim nt of th present inventi n, the associated promoter is a strong and root, root nodule, stem and/or embryo-specific plant promoter such that the SCR protein is overexpressed in the transgenic plant. Examples of root- and root nodules-specific promoters include but are not limited to the promoters of SCR genes, SHR genes, legehemoglobin genes, nodulin genes and root-specific glutamine synthetase genes (See e.g., Tingey et al., 1987, EMBO J. 6:1-9; Edwards et al., 1990, Proc. Nat. Acad. Sci. USA 87:3459-3463).

In yet another preferred embodiment of the present invention, the overexpression of SCR protein in roots may be 15 engineered by increasing the copy number of the SCR gene. One approach to producing such transgenic plants is to transform with nucleic acid constructs that contain multiple copies of the complete SCR gene (i.e., with its own native scr promoter). Another approach is repeatedly transform 20 successive generations of a plant line with one or more copies of the complete SCR gene. Yet another approach is to place a complete SCR gene in a nucleic acid construct containing an amplification-selectable marker (ASM) gene such as the glutamine synthetase or dihydrofolate reductase gene. 25 Cells transformed with such constructs is subjected to culturing regimes that select cell lines with increased copies of complete SCR gene. See, e.g., Donn et al., 1984, J. Mol. Appl. Genet. 2:549-562, for a selection protocol used to isolate of a plant cell line containing amplified copies 30 of the GS gene. Because the desired gene is closely linked to the ASM, cell lines that amplified the ASM gene are also likely to have amplified the SCR gene. Cell lines with amplified copies of the SCR gene can then be regenerated into transgenic plants.

5.3.2. TRANSGENIC PLANTS THAT SUPPRESS ENDOGENOUS SCR EXPRESSION

In accordance with the present invention, a desired plant may be engineered by suppressing SCR activity. In one 5 embodiment, the suppression may be engineered by transforming a plant with a gene construct encoding an antisense RNA or ribozyme complementary to a segment or the whole of SCR RNA transcript, including the mature target mRNA. In another embodiment, SCR gene suppression may be engineered by 10 transforming a plant cell with a gene construct encoding a ribozyme that cleaves the SCR mRNA transcript. Alternatively, the plant can be engineered, e.g., via targeted homologous recombination to inactive or "knock-out" expression of the plant's endogenous SCR.

- For all of the aforementioned suppression constructs, it is preferred that such gene constructs express specifically in the root, root nodule, stem and/or embryo tissues. Alternatively, it may be preferred to have the suppression constructs expressed constitutively. Thus,
- 20 constitutive promoters, such as the nopaline, CaMV 35S promoter, may also be used to express the suppression constructs. A most preferred promoter for these suppression constructs is a SCR or SHR promoter.

In accordance with the present invention, desired 25 plants with suppressed target gene expression may also be engineered by transforming a plant cell with a co-suppression construct. A co-suppression construct comprises a functional promoter operatively associated with a complete or partial SCR gene sequence. It is preferred that the operatively

30 associated promoter be a strong, constitutive promoter, such as the CaMV 35S promoter. Alternatively, the co-suppression construct promoter can be one that expresses with the same tissue and developmental specificity as the scr gene.

According to the present invention, it is preferred 35 that the co-suppressi n construct ncodes a incomplete SCR mRNA, although a construct encoding a fully functional SCR

mRNA or enzyme may also be us ful in effecting cosuppression.

In accordance with the present invention, desired plants with suppressed target gene expression may also be engineered by transforming a plant cell with a construct that can effect site-directed mutagenesis of the SCR gene. (See, e.g., Offringa et al., 1990, EMBO J. 9:3077-84; and Kanevskii et al., 1990, Dokl. Akad. Nauk. SSSR 312:1505-1507) for discussions of nucleic constructs for effecting site-directed mutagenesis of target genes in plants.) It is preferred that such constructs effect suppression of SCR gene by replacing the endogenous SCR gene sequence through homologous recombination with none or inactive SCR protein coding sequence.

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5.3.3. TRANSGENIC PLANTS THAT EXPRESS A TRANSGENE CONTROLLED BY THE SCR PROMOTER

In accordance with the present invention, a desired plant may be engineered to express a gene of interest under the control of the SCR promoter. SCR promoters and functional portions thereof refer to regions of the nucleic acid sequence which are capable of promoting tissue-specific transcription of an operably linked gene of interest in the embryo, stem, root nodule and/or root of a plant. The SCR promoter described herein refers to the regulatory elements of SCR genes as described in Section 5.2.

Genes that may be beneficially expressed in the roots and/or root nodules of plants include genes involved in nitrogen fixation or cytokines or auxins, or genes which regulate growth, or growth of roots. In addition, genes encoding proteins that confer on plants herbicide, salt, or pest resistance may be engineered for root specific expression. The nutritional value of root crops may also be enhanced through SCR promoter driven expression of nutritional proteins. Alt rnatively, therapeutically useful prot ins may be expressed specifically in rot crops.

Genes that may be beneficially xpressed in th stems f plants include thos inv lved in starch lignin or cellul se biosynthesis.

In accordance with the present invention, desired 5 plants which express a heterologous gene of interest under the control of the SCR promoter may be engineered by transforming a plant cell with SCR promoter driven constructs using those techniques described in Section 5.2.2. and 5.3., supra.

10

5.3.4. SCREENING OF TRANSFORMED PLANTS FOR THOSE HAVING DESIRED ALTERED TRAITS

It will be recognized by those skilled in the art that in order to obtain transgenic plants having the desired engineered traits, screening of transformed plants (i.e., those having an gene construct of the invention) having those traits may be required. For example, where the plants have been engineered for ectopic overexpression of SCR gene, transformed plants are examined for those expressing the SCR 20 gene at the desired level and in the desired tissues and developmental stages. Where the plants have been engineered for suppression of the SCR gene product, transformed plants are examined for those expressing the SCR gene product (e.g., RNA or protein) at reduced levels in various tissues. 25 plants exhibiting the desired physiological changes, e.g., ectopic SCR overexpression or SCR suppression, may then be subsequently screened for those plants that have the desired structural changes at the plant level (e.g., transgenic plants with overexpression or suppression of SCR gene having 30 the desired altered root structure). The same principle applies to obtaining transgenic plants having tissue-specific expression of a heterologous gene in embryos and/or roots by the use of a SCR promoter driven expression construct.

Alternatively, the transformed plants may be directly screened for those exhibiting the desir d structural and functi nal changes. In one mbodiment, such scr ening may be for the size, length or pattern of the root of the

transformed plants. In an ther embodim nt, the screening of the transformed plants may be f r altered gravitropism or decreased susceptibility to lodging. In other embodiments, the screening of the transformed plants may be for improved agronomic characteristics (e.g., faster growth, greater vegetative or reproductive yields, or improved protein contents, etc.), as compared to unengineered progenitor plants, when cultivated under various growth conditions (e.g., soils or media containing different amount of nutrients, water content).

According to the present invention, plants engineered with SCR overexpression may exhibit improved vigorous growth characteristics when cultivated under conditions where large and thicker roots are advantageous.

15 Plants engineered for SCR suppression may exhibit improved vigorous growth characteristics when cultivated under conditions where thinner roots are advantageous.

Engineered plants and plant lines possessing such improved agronomic characteristics may be identified by

20 examining any of following parameters: 1) the rate of growth, measured in terms of rate of increase in fresh or dry weight;

2) vegetative yield of the mature plant, in terms of fresh or dry weight; 3) the seed or fruit yield; 4) the seed or fruit weight; 5) the total nitrogen content of the plant; 6) the

25 total nitrogen content of the fruit or seed; 7) the free amino acid content of the plant; 8) the free amino acid content of the fruit or seed; 9) the total protein content of the plant; and 10) the total protein content of the fruit or seed. The procedures and methods for examining these

30 parameters are well known to those skilled in the art.

According to the present invention, a desired plant is one that exhibits improvement over the control plant (i.e., progenitor plant) in one or more of the aforementioned parameters. In an embodiment, a desired plant is one that on parameter. In a pref rred embodiment, a desired plant is ne that shows at least 20% increase over the control plant

in at least one param ter. Most preferred is a plant that shows at least 50% increase in at least one paramet r.

6. EXAMPLE 1: ARABIDOPSIS SCR GENE

This example describes the cloning and structure of the Arabidopsis SCR gene and its expression. The deduced amino acid sequence of the Arabidopsis SCR gene product contains a number of potential functional domains similar to those found in transcription factors. Closely related

10 sequences have been found in both dicots and monocots indicating that Arabidopsis SCR is a member of a new protein family. The expression pattern of the SCR gene was characterized by means of in situ hybridization and by an enhancer trap insertion upstream of the SCR gene (described in more detail in Section 7). The expression pattern is consistent with a key role for Arabidopsis SCR in regulating the asymmetric division of the cortex/endodermis initial which is essential for generating the radial organization of

20

the root.

6.1. MATERIALS AND METHODS

6.1.1. PLANT CULTURE

Arabidopsis ecotypes Wassilewskija (Ws), Columbia (Col), and Landsberg erecta (Ler) were obtained from Lehle.

25 Arabidopsis seeds were surface sterilized and grown as described previously (Benfey et al., 1993, Development 119:57-70). Generation of the enhancer trap lines is described in Section 7.

30 6.1.2. GENETIC ANALYSIS

For the scr-1 allele, co-segregation of the mutant phenotype and kanamycin resistance conferred by the inserted T-DNA was determined as described previously (Aeschbacher et al., 1995, Genes & Development 9:330-340). Because kanamycin affects root growth, 1557 seeds fr m heterozygous lines were germinated on non-s lectiv media, scored for the appearance of the mutant phenotype, and subsequently transferred to

sel ctive m dia. All (284) phenotypically mutant se dlings show d resistance to the antibitic, whereas 834 of 1273 phenotypically wild-type se dlings showed resistance to kanamycin, respectively. Phenotypically wild type plants

- 5 (83) were also transferred to soil and allowed to set seeds. The progeny of these plants were plated on selective and non-selective media, and scored for the co-segregation of the mutant phenotype and antibiotic resistance. A majority (48) of the plants segregated for the mutant phenotype and for
- 10 kanamycin resistance, whereas 35 were wild-type and sensitive to kanamycin. Due to a mis-identified cross, scr-2 was originally thought to be non-allelic and was named pinocchio (Scheres et al., 1995, Development 121:53-62). Subsequent mapping results placed it in an identical chromosomal
- 15 location as scr-1. The original scr-2 line contained at least two T-DNA inserts. Co-segregation analysis revealed a lack of linkage between the antibiotic resistance marker carried by the T-DNA and the mutant phenotype. Antibiotic sensitive lines were identified that segregated for mutants.
- 20 These lines were crossed to scr-1. All F1 antibiotic resistant progeny exhibited a mutant phenotype. All F2 progeny (from independent lines) were mutant, and there was a 3:1 segregation for antibiotic resistance indicating that the two mutations were allelic. Antibiotic sensitive lines of
- 25 scr-2 were found to contain a rearranged T-DNA insert as determined by Southern blots and PCR using T-DNA specific probes and primers respectively. The presence of this T-DNA in the SCR gene was confirmed by Southern blots using SCR probes. A combination of T-DNA and SCR specific primers was
- 30 used to amplify T-DNA/SCR junctions. The PCR fragments were cloned using the TA cloning kit (Invitrogen) and sequenced. The insertion points were determined for both 5' and 3' T-DNA/SCR junctions.

6.1.3. MAPPING

Mutant plants f scr-2 (WS background) were cross d to Col WT. DNA from mutant F2 individual plants wer analyzed for co-segregation with microsatellite (Bell & Ecker, 1994, Genomics 18:137-144) and CAPS markers (Konieczny & Ausubel, 1993, Plant J. 4:403-410). The closest linkage was found to two CAPS markers located at the bottom of chromosome III. Only one out of 238 mutant chromosomes was recombinant for the BGL1 marker (Konieczny & Ausubel, 1993, 10 Plant J. 4:403-410) and one out of 210 chromosomes was recombinant for the cdc2b marker.

A RFLP for the SCR gene was identified between Col and Ler ecotypes with Xho I endonuclease. Genomic DNAs from independent R1 lines (Jarvis et al., 1994, Plant Mol. Biol.

15 24:685-687) were digested with Xho I and blots were hybridized to SCR. Using the segregation data obtained for 25 R1 lines, the SCR gene was mapped relative to molecular markers by CLUSTER. The SCR gene was assigned to the bottom of chromosome III closest to BGL1.

20

6.1.4. PHENOTYPIC ANALYSIS

Morphological characterization of the mutant roots was performed as follows: 7 to 14 days post-germination phenotypically mutant seedlings were fixed in 4.0%

- 25 formaldehyde in PIPES buffer pH 7.2. After fixation the samples were dehydrated in ethanol followed by infiltration with Historesin (Jung-Leica, Heidelberg, Germany). Plastic sections were mounted on superfrost slides (Fisher). The sections were either stained with 0.05% toluidine blue and
- 30 photographed using Kodak 160T film or used for Casparian strip detection or antibody staining.

Casparian strip detection was performed as described previously (Scheres et al., 1995, Development 121:53-62), with the following modifications. Plastic 35 sections were us d and the cunterstaining was done in 0.1% aniline blu for 5 to 15 min. The sections were visualized with a Leitz fluor sc nt microscope with FITC filter.

Pictur s w r tak n using a Leitz cam ra attached to the microscope and Kodak HC400 film. Slides were digitized with a Nikon slide scanner and manipulated in Adobe Photoshop.

For antibody staining, sections were blocked for 2 5 hours at room temperature in 1% BSA in PBS containing 0.1% Tween 20 (PBT). Samples were incubated with primary antibodies at 4° C in 1% BSA in PBT overnight, and then washed 3 times 5 minutes each with PBT. Samples were incubated for two hours with biotinylated secondary

- 10 antibodies (Vector Laboratories) in PBT, and washed as above. Samples were incubated with Texas Red conjugated avidin D for 2 hours at room temperature, washed as before, and mounted in Citifluor. Immunofluorescence was observed with a fluorescent microscope equipped with a Rhodamine filter.
- 15 Staining with the CCRC antibodies was performed as described previously (Freshour et al., 1996, Plant Physiol. 110:1413-1429).

6.1.5. MOLECULAR TECHNIQUES

- Genomic DNA preparation was performed using the Elu-Quik kit (Schleicher & Schuell) protocol. Radioactive and non-radioactive DNA probes were labeled with either random primed labeling or PCR-mediated synthesis according to the Genius kit manual (Boehringer Mannheim). E. coli and
- 25 Agrobacterium tumefaciens cells were transformed using a BIO-RAD gene pulser. Plasmid DNA was purified using the alkaline lysis method (Maniatis et al., Molecular Cloning: A Laboratory Manual, Cold Spring Harbor, New York: Cold Spring Harbor Laboratory, 1982).
- A probe made from a rescued fragment of 1.2 kb was used to screen a wild-type genomic library made from WS plants. One genomic clone containing an insert of approximately 23 kb was isolated. A 3.0 kb Sac I fragment from the genomic clone, which hybridized to the 1.2 kb probe,
- 35 was subclon d and sequenced (FIG. 5A). Comparison of the nucl otide s qu nc betw n th g nomic clone and th rescu d plasmid r v aled the site of th T-DNA insertion.

Appr ximately 600,000 plaques from a cDNA library, obtained from inflorescences and siliques (Col ecotype), and ther fore enriched in embryos, were screened with the 1.2 kb probe. Four cDNA clones were isolated. The dideoxy sequencing 5 method was performed using the Sequenase kit (United States Biochemical Corp.). Sequence-specific internal primers were synthesized and used to sequence the Sac I genomic as well the cDNA clones. Total RNA from plant tissues was obtained using phenol/chloroform extractions as described in (Berry et al., 1985, Mol. Cell. Biol. 5:2238-2246) with minor modifications. Northern hybridization and detection were performed according to the Genius kit manual (Boehringer Mannheim).

To identify the site of insertion of the enhancer15 trap T-DNA, genomic DNA from ET199 homozygous plants was amplified using primers specific for the T-DNA left border and the SCR gene. An approximately 2.0 kb fragment was amplified. This fragment was sequenced and the site of insertion was found to be approximately 1 kb from the ATG 20 start codon.

6.1.6. IN SITU HYBRIDIZATION

Antisense and sense SCR riboprobes were labeled with digoxigenin-11-UTP (Boehringer Mannheim) using T7
25 polymerase following the manufacturer's protocol. Probes contained a 1.1 kb 3' portion of the cDNA. Probe purification, hydrolysis and quantification were performed as described in the Boehringer Mannheim Genius System user's guide.

Tissue samples were fixed in 4 % formaldehyde overnight at 4°C and rinsed two times in PBS (Jackson et al., 1991, Pl. Cell 3:115-125). They were subsequently preembedded in 1 % agarose in PBS. The fixed tissue was dehydrated in ethanol, cleared in Hemo-De (Fisher Scientific, 35 Pittsburgh, PA) and emb dd d in ParaplastPlus (Fisher Sci ntific). Tissue s cti ns (10µm thick) w re mounted on Superfr stPlus slides (Fish r Scientific). S ction

pretreatment and hybridization were p rformed according to (Lincoln et al., 1994, Plant Cell 6:1859-1876) xc pt that proteinas K was used at 30 mg/ml and a two h ur prehybridization step was included. Probe concentration of 5 0 ng/ml/kb was used in the hybridization.

Slides were washed and the immunological detection was performed according to (Coen et al., 1990, Cell 63:1311-1322) with the following modifications. Slides were first washed 5 h in 5xSSC, 50% formamide. After RNase treatment 10 slides were rinsed three times (20 min each) in the buffer (0.5 M NaCl, 10 mM Tris-HCl pH 8.0, 5.0 mM EDTA). In the immunological detection, antibody was diluted 1:1000, levamisole (240 ng/ml) was included in the detection buffer, and after stopping the reaction in 10 mM Tris, 1 mM EDTA, 15 sections were mounted directly to Aqua-Poly/Mount (Polysciences, Warrington, PA).

6.2. RESULTS

6.2.1. CHARACTERIZATION OF THE SCR PHENOTYPE

The scarecrow mutant scr-1 was isolated in a screen 20 of T-DNA transformed Arabidopsis lines (Feldmann, K.A., 1991, Plant J. 1:71-82), as a seedling with greatly reduced root length compared to wild-type (Scheres et al., 1995, Development 121:53-62). A second mutant scr-2 with a similar 25 phenotype was subsequently identified among T-DNA transformed lines. Analysis of co-segregation between the mutant phenotype and antibiotic resistance carried by the T-DNA indicated tight linkage for scr-1 and no linkage for scr-2 (see Experimental Procedures). An antibiotic sensitive line 30 of scr-2 was isolated and crossed with scr-1. The F2 progeny of this cross were all mutant and segregated 3:1 for antibiotic resistance confirming allelism (see Materials & Methods). The principal phenotypic difference between the two alleles was that scr-1 root growth was more retarded than 35 that of scr-2, suggesting that it is the stronger allele (FIG. 2A). F r both all les th a rial organs appeared similar t wild-type and th flowers were fertil (FIGS. 2A

and 2B). The progeny of backer sses of scr-1 r scr-2 to wild-type plants segregated 3:1 for the root phenotype f r both allel s, indicating that each mutation is monogenic and recessive.

- Analysis of transverse sections through the primary root of seedlings revealed only a single cell layer between the epidermis and the pericycle (FIG. 2C) instead of the normal radial organization consisting of cortex and endodermis (FIG. 2D). This radial organization defect was 10 not limited to the primary root, but was also present in secondary roots (FIG. 2E) and in roots regenerated from calli (FIG. 2F). Occasionally defects were observed in the number of cells in the remaining cell layer (more than the invariant 8 found in wild-type). Abnormal placement or numbers of 15 epidermal cells were also observed (see FIG. 2E). abnormalities were more frequently observed in scr-1 than in scr-2. Nevertheless, organization of the mutant root closely resembles that of wild-type except for the consistent reduction in the number of cell layers. Because the 20 endodermis and cortex are normally generated by an asymmetric division of the cortex/endodermal initial, this indicates that the primary defect in scr is disruption of this
- It has been shown that the radial organization

 25 defect in scr-1 first appears in the developing embryo at the early torpedo stage and manifests itself as a failure of the embryonic ground tissue to undergo the asymmetric division into cortex and endodermis (Scheres et al., 1995, Development 121:53-62). This defect extends the length of the embryonic axis which encompasses the embryonic root and hypocotyl. Other embryonic tissues appear similar to wild-type (Scheres et al., 1995, Development 121:53-62). In seedling hypocotyls of the scarecrow phenotype, two cell layers instead of the normal three layers (two cortex and one endodermis) between 35 epidermis and stel were found. This w uld be the exp cted result f th lack f the divisi n of th embryonic ground tissue. Similar results were btained for scr-2. Henc,

asymmetric division.

this mutant identifies a gene involved in the asymmetric division that pr duc s cortex and ndod rmis from ground tissue in the embryonic r t and hypocotyl and from the cortex/endodermal initials in primary and secondary roots.

5

6.2.2. CHARACTERIZATION OF CELL IDENTITY IN SCR

in regulating this asymmetric division, it was necessary to determine the identity of the mutant cell layer. Tissue-specific markers were used to distinguish between several possibilities. The cell layer could have differentiated attributes of either cortex or endodermis. Alternatively, it could have an undifferentiated, initial-cell identity or it could have a chimeric identity with differentiated attributes of both endodermis and cortex in the same cell.

Transverse sections of scr-1 and scr-2 roots were assayed for the presence of tissue-specific markers. casparian strip, a deposition of suberin between radial cell 20 walls, is specific to the endodermal cells and is believed to act as a barrier to the entry of solutes into the vasculature (Esau, K. Anatomy of Seed Plants, New York: John Wiley & Sons, 1977, Ed. 2, pp. 1-550). Histochemical staining revealed the presence of a casparian strip in the mutant cell layer (FIG. 3A, compare to wild-type, FIG. 3B). It is noted that in the vascular cylinder, this histochemical stain also reveals the presence of lignin, indicating the presence of differentiated xylem cells in mutant (FIG. 3A) and wild-type (FIG. 3B). Another marker of the differentiated endodermis 30 is the arabinogalactan epitope recognized by the monoclonal antibody, JIM13 (Knox et al., 1990, Planta 181:512-521). The mutant cell layer showed staining with this antibody (FIG. 3C, compare with wild-type, FIG. 3B). As a positive control, the JIM7 antibody that recognizes pectin epitopes in all cell walls was used (FIGS. 3E and 3F). These results indicat that the cell lay r between th epid rmis and th pericycle has differentiated attributes of the endodermis.

As a marker for th cortex, the CCRC-M2 monoclonal antibody was used. This antibody recogniz s a c ll wall oligosaccharide pitop, f und only n differentiated cortex and epidermis cells. In sections from the differentiation 5 zone of scr-1 and scr-2, both cortex and epidermal cells showed staining (FIG. 4A and 4B) that was similar to that of wild-type (FIG. 4C). In scr-1, staining of both cell types was apparent, but staining of cortex was somewhat weaker than wild-type. The positive control used the CCRC-M1 monoclonal antibody which recognizes an oligosaccharide epitope found on all cells (FIGS. 4D-F).

With the CCRC-M2 antibody an interesting difference was observed between the staining pattern of the mutants as compared to wild-type. The appearance of this epitope 15 correlates with differentiation in these two cell types. Normally, in sections close to the root tip there is no staining. In sections higher up in the root, atrichoblasts (epidermal cells that do not make root hairs) stain. sections from more mature root tissue, all epidermal cells as 20 well as cortex cells stain for this epitope. In both scr-1 and scr-2, sections could be found in which all epidermal cells stained while there was little detectable staining of cortex cells. Although not precisely identical to the wildtype staining pattern, the fact that the mutant cell layer 25 clearly stains for this cortex marker indicates that there are cortex differentiated attributes expressed in these cells.

Taken together, these results indicate that the mutant cell layer has differentiated attributes of both the 30 endodermis and cortex. The possibility that there has been a simple deletion of a cell type, or that the resulting cell type remains in an undifferentiated initial-like stage can be ruled out. This result is consistent with a role for the scr gene in regulating this asymmetric division rather than a 35 role in directing c ll sp cification.

6.2.3. MOLECULAR CLONING OF THE SCR GENE

To furth r lucidat th function of th Arabid psis SCR gen th insert d T-DNA s quences were used to clone the gene. Plant DNA flanking the insertion site was 5 obtained from scr-1 by plasmid rescue and used to isolate the corresponding wild-type genomic DNA. Several cDNA clones were isolated from a library made from silique tissue. Comparison of the sequence of the longest cDNA and the corresponding genomic region revealed an open reading frame 10 (ORF) interrupted by a single small intron. (FIG. 5A). A potential TATA box and polyadenylation signal that matched the consensus sequences for plant genes were also identified (Joshi, C.P., 1987, Nucl. Acids Res. 15:6643-6653); Heidecker & Messing, 1986, Ann. Rev. Plant Physiol. 37:439-466); Mogen 15 et al., 1990, Plant Cell 2:1261-1272).

Comparison of the nucleotide sequence between the genomic clone and the rescued plasmid placed the site of the T-DNA insertion in scr-1 at codon 470 (FIGS. 5A and 5B). For scr-2, although no linkage was found between the mutant

- 20 phenotype and antibiotic resistance, DNA blot and PCR analysis of antibiotic sensitive lines revealed the presence of T-DNA sequences that co-segregated with the mutant phenotype. The insertion position in scr-2 was determined by cloning and sequencing the PCR products amplified from its
- 25 genomic DNA using a combination of T-DNA and SCR specific primers at both sides of the insertion (FIG. 5B). In scr-2 the T-DNA insertion point is at codon 605 (FIG. 5A and 5B). To verify linkage between the cloned gene and the mutant phenotype, we identified the chromosomal location of both the
- 30 scr locus and the SCR gene. To map the scr locus, molecular markers were used on F2 progeny of crosses between scr-2 (ecotype Wassilewskija, Ws) and Colombia (Col) WT. These placed the scr locus at the bottom of chromosome III, approximately 0.5 cM away from each of the two closest
- 35 markers available, cdc2b and BGL1 (Konieczny and Ausubel, 1993, Plant J. 4:403-410). To map th SCR g ne, we identified a polymorphism betw en Col and Landsberg (Ler)

cotypes using the SCR probe b (FIG. 5B). South rn analysis of 25 recombinant inbred lin s (Jarvis t al., 1994, Plant Mol. Biol. 24:685-687) mapped th cl n d gene to the sam location as the SCR locus on chromosome III.

The determination of the molecular defects in two independent alleles and the co-localization of the cloned gene and the mutant locus confirms that we have identified the SCR gene.

10 6.2.4. THE SCR GENE HAS MOTIFS THAT INDICATE IT IS A TRANSCRIPTION FACTOR

The Arabidopsis SCR gene product is a 653 amino acid polypeptide that contains several domains (FIG. 5B). The amino-terminus has homopolymeric stretches of glutamine, 15 serine, threonine, and proline residues, which account for 44% of the first 267 residues. Domains rich in these residues have been shown to activate transcription and may serve such a role in SCR (Johnson et al., 1993, J. Nutr. Biochem 4:386-398). A charged region between residues 265 20 and 283 has similarity to the basic domain of the bZIP family of transcriptional regulatory proteins (FIG. 5C) (Hurst, H.C., 1994, Protein Profile 1:123-168). The basic domains from several bZIP proteins have been shown to act as nuclear localization signals (Varagona et al., 1992, Plant Cell 25 4:1213-1227), and this region in SCR may act similarly. charged region is followed by a leucine heptad repeat (residues 291-322). A second leucine heptad repeat is found toward the carboxy-terminus (residues 436 to 473). leucine heptad repeats have been demonstrated to mediate 30 protein-protein interactions in other proteins (Hurst, H.C., 1994, Protein Profile 1:123-168), the existence of these motifs suggests that SCR may function as a dimer or a multimer. The second leucine heptad repeat is followed by a small region rich in acidic residues, also present in a 35 number of defin d transcriptional activation d mains (Johns n et al., 1993, J. Nutr Biochem 4:386-398). While each of these domains has been found within proteins that do not act

as transcriptional regulat rs, the fact that all of them are found within the deduced SCR protein sequence indicates that SCR is a transcriptional regulatory prot in.

5 6.2.5. SCR IS A MEMBER OF A NOVEL PROTEIN FAMILY The Arabidopsis SCR protein sequence was compared with the sequences in the available databases. expressed sequence tags (ESTs), nine from Arabidopsis, one from rice and one from maize, showed significant similarity 10 to residues 394 to 435 of the SCR sequence, a region immediately amino-terminal to the second leucine heptad repeat (FIGS. 15K-L). This region is designated the VHIID Subsequent analysis of these EST sequences has revealed that the sequence similarity extends beyond this 15 region; in fact, the similarity extends throughout the entire known gene products. The combination and order of the motifs found in these sequences do not show significant similarity to the general structures of other established regulatory protein families (i.e., bZIP, zinc finger, MADS-domain, and 20 homeodomain), indicating that the SCR proteins comprise a novel family.

6.2.6. SCR IS EXPRESSED IN THE CORTEX/ENDODERMAL INITIALS AND IN THE ENDODERMIS

25 RNA blot analysis revealed expression of SCR in Arabidopsis siliques, leaves and roots of wild-type plants (FIG. 6A). No hybridization was detected to RNA from scr-1 plants (FIG. 6B, lane 2). This indicates that scr-1 has a reduced level of RNA expression and may represent the null phenotype. Hybridization to RNA species larger than the normal size were detected in scr-2. This indicates that abnormal SCR transcripts are made in this allele, suggesting that functional but possibly altered proteins may be produced.

To determine if expression was localized to any particular c ll type, RNA in situ was hybridization perf rmed on sections of root tissue. In mature roots, expression was

localized primarily to the endodermis (FIGS. 7A and 7B).

Expression appear d to start very cl s to or within the cortex/ ndodermal initials and continue up the endodermal cell file as far as the section extended. Expression was also detected in late-torpedo stage embryos in the endodermis throughout the embryonic axis (FIG. 7C). Sense strand controls showed only background hybridization (FIG. 7D).

To determine whether the localization of SCR RNA was regulated at the transcriptional or post-transcriptional level, enhancer trap (ET) lines were prepared and examined in which the β-glucuronidase (uid-A or GUS) coding sequence with a minimal promoter was expressed in the root endodermis. (See Section 7, infra). Restriction fragment length polymorphisms were observed when DNA from one of these lines, ET199 and wild-type were probed with SCR. PCR and sequence analysis confirmed that the enhancer-trap construct had inserted approximately 1 kb upstream of the SCR start site and in the same orientation as that of SCR transcription.

In mature roots, expression in ET199 whole mounts

20 showed a similar pattern to that of the in situ
hybridizations, with the strongest staining present in
endodermal cells (FIG. 7E). Transverse sections indicated
that expression was primarily in endodermal cells in the
elongation zone (FIG. 7F). Longitudinal sections through the

25 meristematic zone revealed that expression could be detected
in the cortex/endodermal initial (FIG. 7G). Of particular
interest was the restriction of expression to the endodermal
daughter cell after the periclinal division (FIG. 7G). This
indicated that the expression pattern observed in the in situ

30 analysis was not due to post-transcriptional partitioning of
SCR RNA. Rather, it suggests that after the periclinal
division of the cortex/endodermis initial only one of the two
cells is able to transcribe SCR RNA.

6.3. DISCUSSION

6.3.1. THE SCR GENE REGULATES AN ASYMMETRIC DIVISION REQUIRED FOR ROOT RADIAL ORGANIZATION

The formation of the cortex and endodermal layers 5 in the Arabidopsis root requires two asymmetric divisions. In the first, an anticlinal division of the cortex/endodermal initial generates two cells with different developmental potentials. One will continue to function as an initial, while the other undergoes a periclinal division to generate the first cells in the endodermal and cortex cell files. This second asymmetric division is eliminated in the scarecrow mutant, resulting in a single cell layer instead of The scr mutation appears to have little effect on any other cell divisions in the root indicating that it is involved in regulating a single asymmetric division in this Several other mutations have been characterized that appear to affect specific cell division pathways in Arabidopsis. These include knolle (kn) in which formation of the epidermis is impaired (Lukowitz et al., 1996, Cell 84:61-71), wooden leg (wol) in which vascular cell division is defective (Scheres et al., 1995, Development 121:53-62) and fass (fs) in which there are supernumerary cortex and vascular cells (Scheres et al., 1995, Development 121:53-62); Torres Ruiz & Jurgens, 1994, Development 120:2967-2978). Only in the case of scr and short-root (shr) mutants has it been shown that the defect is in a specific asymmetric division.

revealed that the genes that regulate asymmetric divisions can be specific to a single type of division or can affect divisions that are not clonally related (Horvitz & Herskowitz, 1992, Cell 68:237-255). In most cases, these mutations result in the formation of two identical daughter cells with similar developmental potentials (Horvitz & Herskowitz, 1992, Cell 68:237-255). Both resulting c lls have the identity of one or the other of the normal daughter

cells, an example of which is the swi mutation in S. c revisiae (Nasmyth et al., 1987, Cell 48:579-587). However, ther are also xampl s of mutations that result in the formation of chimeric cell types such as the ham-1 mutation 5 in C. elegans (Desai et al., 1988, Nature 336:638-646).

6.3.2. SCR INVOLVEMENT IN CELL SPECIFICATION OR CELL DIVISION

Genes that regulate asymmetric cell divisions can 10 be divided into those that specify the differentiated fates of the daughter cells and those that function to effect the division of the mother cell (Horvitz & Herskowitz, 1992, Cell, 68:237-255). The aberrant cell layer formed in the scrmutant has differentiated features of both endodermal and 15 cortex cells. Thus, scr is in the rare class of asymmetric division mutants in which a chimeric cell type is created. The ability to express differentiated characteristics of cortex and endodermal cells implies that the differentiation pathways for both these cell types are intact and do not 20 require the functional SCR gene. This indicates that SCR is involved primarily in regulating a specific cell division, and that the correct occurrence of this division can be unlinked from cell specification. This is in contrast to the shr mutant, in which the periclinal division of the 25 cortex/endodermal initial also fails to occur and the resulting cell lacks endodermal markers (Benfey et al., 1993, Development 119:57-70) and has cortex attributes. A genetic analysis was used to address the function of SHR and SCR in the asymmetric division of the cortex/endodermal initial. 30 Placing mutants of each of these genes in a fs mutant background asked whether the supernumerary cell divisions characteristic of fs were sufficient to restore normal cell identities (Scheres et al., 1995, Development 121:53-62).

the shr,fs double mutant there were additional cell layers

35 but no endodermal, indicating that th SHR g n has a role in specifying c ll id ntity. In th scr,fs doubl mutant no alterati n in c ll id ntity was observed as compared to fs

(Scher s t al., 1995, Dev lopment 121:53-62). Taken together with th cell marker analysis present d herein, these results ar c nsist nt with a role for SCR in generating the division of the mother cell while the SHR gene may be involved in specifying the fate of the endodermal daughter.

6.3.3. A ROLE FOR SCR IN EMBRYONIC DEVELOPMENT

At least one additional cell division appears to be 10 affected in the scr mutant. During embryonic development, the ground tissue does not divide to form the endodermal and cortex layers of the embryonic root and hypocotyl. As shown herein, expression of SCR was detected in the endodermal tissue throughout the embryonic axis shortly after this

- 15 division occurs. Thus, SCR may play a direct role in regulating both this division and the division of the cortex/endodermal initial in the root apical meristem. Alternatively, the radial organization established in the embryo may somehow act as a template that directs the
- 20 division of the cortex/endodermal initial, thus perpetuating the pattern. This is consistent with the finding in the scr mutant that the aberrant pattern established in the embryo is perpetuated in the primary root. It is also consistent with a recent study in which the daughter cells of the
- 25 cortex/endodermal initial were laser ablated (van den Berg et al., 1995, Nature 378:62-65). When a single daughter cell was ablated, it was replaced by a cell that followed the normal asymmetric division pattern. When three adjacent daughter cells were ablated, the central initial divided
- 30 anticlinally but failed to perform the periclinal division (van den Berg et al., 1995, Nature 378:62-65). This provided evidence that information from mature cells is required for the correct division pattern of cortex/endodermal initials suggesting a "top down" transfer of information. However,
- 35 the abs nce of a cell lay r in lateral roots and callusd rived r ots of the scr mutant sugg sts that embryo vents are not unique in their ability to establish radial

organization. Rath r, these bservati ns implicate SCR in regulating both embryonic and p st- mbryonic r ot radial organization.

5 6.3.4. TISSUE-SPECIFIC EXPRESSION OF SCR IS REGULATED AT THE TRANSCRIPTIONAL LEVEL

Although not intending to be limited to any theory or explanation regarding the mechanism of SCR action, the cloning of the gene and the expression pattern provide some 10 clues as to the role of SCR in the regulation of a specific asymmetric division. The SCR gene is expressed in the cortex/endodermal initial, but immediately after division is restricted to the endodermal lineage. A similar pattern is seen in the ET199 enhancer trap line in which SCR regulatory 15 elements are in proximity to a GUS gene, indicating that SCR restriction to the endodermal cell file is due to differential regulation of expression of the SCR gene in this cell and the first cell in the cortex file. Another marker line in which expression of GUS is detected only in the 20 cortex daughter cell provides a control for differential degradation of GUS RNA or protein. Thus, partitioning of SCR RNA as a means of achieving this segregation of expression can be ruled out. What remains to be determined is whether this difference in transcriptional activity of the two 25 daughter cells is due to internal polarity of the mother cell prior to division such that cytoplasmic determinants are unequally distributed, or to external polarity that influences cell fate after division. Since SCR is expressed prior to cell division, an attractive hypothesis is that it 30 is involved in establishing polarity in the cortex/endodermal initial. The sequence of the SCR protein strongly suggests that it acts as a transcription factor. Hence, it may act to regulate the expression of other genes essential for the establishment of unequal division. Alternatively, it is 35 conceivable that it could play a role in creating an ext rnal polarity that provid s a signal to divid asymmetrically.

Its xpr ssion in mor mature ndodermal c lls is consistent with a rol in "top-down" signaling.

6.3.5. A NEW FAMILY OF TRANSCRIPTIONAL REGULATORS

- Analysis of eighteen EST clones found in the 5 GenBank database reveals that the proteins they encode share a high degree of homology with Arabidopsis SCR protein. Table 1 and FIGS. 15A-S. Further sequence analysis of the encoded proteins indicate that a high degree of sequence 10 similarity extends from at least the highly conserved VHIID domain to the carboxy-terminus of the gene products. Comparison of the amino termini of these proteins is precluded by the fact that the ESTs are incomplete. The high degree of similarity among these proteins, in combination 15 with the motifs observed in the SCR protein (homopolymeric motifs, two leucine heptad repeats and a bZIP-like basic domain that may also function as a nuclear localization sequence) indicates that these proteins form a novel class of regulatory proteins.
- mutant alleles raised the possibility that the mutant phenotype was due to the production of truncated proteins. Northern blot analysis indicated SCR RNA is undetectable in scr-1. This suggests that the phenotype is either the null, 25 or due to highly reduced RNA expression. In scr-2, an alteration in RNA size was detected which would be consistent with the presence of a functional and possibly truncated protein. This could provide an explanation for the observation that scr-2 appears to be the weaker allele.

30

7. EXAMPLE 2: ENHANCER TRAP ANALYSIS OF ROOT DEVELOPMENT

An enhancer trap system was used in order to provide a more detailed molecular analysis of gene expression in lateral root patterning and development in Arabidopsis

35 thaliana. A new collection of marker lines that express β-glucuronidase (GUS) activity in a c ll-type specific manner in each of the cells f the root was gen rat d. These lines

allow differentiation of c lls to be monitor d based on molecular charact ristics. One of th se mark r lines, ET199, resulted from th integrati n of th GUS cassette in proximity to an SCR enhancer. The results described below demonstrate that transcriptional activation of the SCR gene plays an important role in root development in Arabidopsis, and that SCR gene transcriptional regulatory elements can express a transgene in a developmentally and tissue specific manner.

10

7.1. MATERIALS AND METHODS

7.1.1. PLANT GROWTH CONDITIONS:

Arabidopsis seeds from NO-O and Columbia ecotypes were sterilized and sown on MS plates containing 4.5%

15 sucrose. Plates were oriented vertically and maintained under 18 hours light, 6 hours dark cycle.

7.1.2. HISTOLOGY AND GUS STAINING:

For observation of lateral roots, roots were
20 removed from plates and infiltrated in 25% glycerol for
several hours to overnight. Roots were then mounted in 50%
glycerol. Whole seedlings were stained for GUS activity for
up to three days in the following solution: 1X GUS buffer,
20% methanol, 0.5 mg/ml X-Glu. Addition of methanol greatly

- 25 improves the specificity and reproducibility of staining. Staining solution was made fresh from a 10X buffer (1 M Tris pH7.5, 290 mg NaCl, 66 mg K₃Fe(CN)₆) that was stored for no more than one week. Stained roots were cleared in glycerol and mounted as above. All samples were observed using
- 30 Nomarski optics on a Leitz Laborlux S microscope.

 Photographs were taken using a Leitz MPS52 camera, and images were scanned into Adobe Photoshop to create figures. In some cases the intensity of the blue color was increased.

7.1.3. CONSTRUCTION OF ENHANCER TRAP LINES:

Plant Cloning V ct r (PCV) (Koncz et al., 1994, Specialized v ctors for g n tagging and expr ssion studies, in Plant Molecular Biology Manual, Gelvin & Schilperoort, 5 eds., Vol. B2, pp. 1-2, Kluover Academic Press, Dordrecht, The Netherlands) contains a Bam HI site immediately adjacent to the T-DNA right border sequence. The β-glucuronidase gene fused to the TATA region (-46 to 78) of the CaMV 35S promoter was introduced into this site (Benfey et al., 1990, EMBO J. 9:1677-1684). 350 transgenic lines were generated by Agrobacterium mediated root transformation (Marton & Browse, 1991, Plant Cell Reports 10:235-239), and 4 independent lines from each transformant were screened for GUS activity in the

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root.

7.2. RESULTS

7.2.1. DIFFERENTIATION IN THE LRP

The marker lines described above reflect patterns of gene expression that are specific to individual root cell types. There are no readily apparent mutant phenotypes in any of these lines. Therefore, they can be used to analyze the differentiation state of the cells during normal development of the lateral root primordial (LRP). If there are stages at which the pericycle cells proliferate in the 25 absence of patterning, it can be expected that all cells would be identical with none expressing differentiated characteristics. In contrast, organization of the LRP would be reflected in differential patterns of GUS gene expression, with certain cells beginning to turn on transcription from 30 differentiated cell-type specific promoters (i.e., those that drive GUS expression in the enhancer trap lines).

The process of lateral root formation is divided into the following seven stages:

Stag I: The LRP is first visible as a set of pericycle cells that are clearly short r in 1 ngth than their neighbors, having undergone a series of anticlinal divisions. Laskowski et al., 1995, Dev. 121:3303-3310 predict that there are approximately 4 founder pericycle cells involved. In the longitudinal plane, these divisions result in the formation of 8-10 small cells, which enlarge in a radial direction.

Stage II: A periclinal division occurs that divides the LRP into two layers (Upper Layer (UL) and Lower Layer (LL)). Not all the small pericycle-derived cells appear to participate in this division -- typically the most peripheral cells do not divide. Hence, as the UL and LL cells expand radially the domed shape of the LRP begins to appear.

15

Stage III: The UL divides periclinally, generating a three layer primordium comprised of UL1, UL2 and LL. Again, some peripheral cells do not divide, creating peripheral regions that are one and two cell layers thick. This further 20 emphasizes the domed shape of the LRP.

Stage IV: The LL divides periclinally, creating a total of four cell layers (UL1, UL2, LL1, LL2). At this stage the LRP has penetrated the parent endodermal layer.

25

Stage V: The central cells in LL2 undergo a number of divisions that push the overlying layers up and distort the cells in LL1. These divisions are difficult to visualize at this stage, but clearly form a knot of mitotic activity. The 30 LRP at this stage is midway through the parent cortex. The outer layer contains 10-12 cells.

Stage VI: This stage is characterized by several events.
The four central cells of UL1 divide periclinally. This

35 divisi n is particularly useful in identifying th median longitudinal plane in th enlarging LRP. At this point th re are a total of twelve cells in UL1, four in the middle

that hav undergon the priclinal division and four on eith r sid. In addition, all but the most central cells of UL2 undergo a periclinal division. At this point the LRP has passed through the parent cortex layer and has penetrated the epidermis. The central cells apparently derived from LL2 have a distinct elongated shape characteristic of vascular elements.

Stage VII: As the primordium enlarges it becomes difficult to characterize the divisions in the internal layers. However, the cells in the outermost layer can still be seen very clearly. All of these cells undergo a anticlinal division, resulting in 16 central cells (8 cells in each of two layers) flanked by 8-10 cells on each side. We refer to 15 this as the 8-8-8 cell pattern. The LRP appears to be just about to emerge from the parent root.

7.2.2. MARKER LINES

An enhancer trapping cassette was generated by 20 fusing the GUS coding sequence to the minimal promoter of the 35S promoter from CaMV. This minimal promoter does not produce a detectable level of GUS expression. However, its presence allows other upstream elements to direct GUS expression in a developmental and/or cell-specific manner 25 (Benfey et al., 1990, EMBO J. 9:1677-1684). The use of a minimal promoter instead of a promoterless construct allows GUS expression to occur even if the enhancer trap cassette inserts at a distance from the coding region. Since the insert does not have to be within the structural gene, there 30 are often no mutations generated in the enhancer trap lines. The minimal promoter: GUS construct was cloned immediately adjacent to the T-DNA right border sequence of PCV (Koncz et al., supra) and introduced into Arabidopsis. 350 independent lines were generated and analyzed for GUS activity in the 35 root. The following lines most cl arly defin each c ll All of the lines wer generated through enhancer trapping, as described herein, below, except for CorAX92

(Dietrich et al., 1992, Plant Cell 4:1371-1382) and EpiGL2:GUS (Masucci et al., D v. 122:1253-1260) which ar transgenic plants that contain cell-type sp cific promoters fused to the GUS gene.

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Ste05 - expresses GUS in the stele including the pericycle layer throughout primary and lateral roots. At the root tip, staining becomes weaker in the elongation zone; therefore, it is likely that only differentiated stele cells express GUS 10 activity. Stelar GUS expression is also seen in aerial parts of the plant.

End195 - expresses GUS in the endodermis of primary and lateral roots. Staining can be seen most clearly in the 15 cells in the meristematic region of the root, although overstaining shows that more mature cells also express some GUS activity. It appears that there is no staining in the cortex/endodermal initial, but staining is evident in the first daughter cell of this initial. GUS expression is also seen at the base of young leaves and in the stipules.

ET199 - expresses GUS in the endodermis of primary and lateral roots, again most clearly in cells in the meristematic region. Unlike End195, staining in ET199
25 appears to continue down to the cortex/endodermal initial and, in younger roots, even into the cells of the quiescent center. Expression in the aerial parts of the plant is detectable in the young leaf primordia.

- 30 CorAX92 This line was generated by fusing the 5' and 3' sequences from a cortex specific gene isolated from oilseed rape to the GUS reporter gene (Dietrich et al., Plant Cell 4:1371-1382). Expression is limited to the cortex layer, extending to but not including the cortex/endodermal initial.
- 35 Staining is also apparent in th petioles and leaf blad s of expanded leaves.

EpiGL2:GUS - This line was g n rated by fusing the GL2
promoter to the GUS gene (Masucci et al., Dev. 122:12531260). Expression is seen in the non-hair forming epidermal
cells (atrichoblasts). Staining is seen near the root tip,
but it is difficult to determine if it includes the epidermal
initial. Staining is also seen in the trichomes, leaf
primordia, and the epidermis of the hypocotyl and leaf
petioles.

10 CRC219 - This line shows staining in the columella root cap only.

LRC244 - This line shows staining in the lateral root cap only.

15

RC162 - This line shows staining in both the lateral and columella root caps.

Two marker lines show differential staining at

20 very early stages of LRP development. One of these, ET199,
presents a complex and dynamic pattern of expression.

Staining is first apparent at stage II in only the four
central cells of the UL. At stage III staining is strongest
in the central cells of UL2. As the LRP reaches stage V the

25 staining remains strongest in the central 2-4 cells of UL2.

By stage VI staining also begins to extend into the newly
formed endodermal layer, and staining in both the central
cells and endodermis persists beyond emergence of the lateral
root.

- Another line, LRB10 (lateral root base), does not express GUS in the primary root tip. Staining in the LRP is seen at stage I, and at stage II all the cells of the UL and LL are stained. However, by stage IV and V only the cells at the periphery of the LRP are still expressing GUS. As the
- 35 LRP dev lops, th se cells continue to stain, although less intens ly, resulting in a ring of GUS xpressing cells at the base of the LR.

LRB10 and ET199 clearly demonstrate non-identity between the colls at very arranges, stage IV in the cas of LRB10 and within the UL at stage II in ET199. In addition, although it is difficult to identify the nature of the cells that correspond to the observed staining pattern in LRB10 and the early staining cells of ET199, post-emergent lateral roots show analogous staining in these lines, suggesting that the stained cells are already expressing markers that reflect their differentiated cell fates. Hence, these observations suggest a very early onset of differentiation in the cells of the LRP.

7.2.3. ET199 PROVIDES EVIDENCE FOR THE ROLE OF SCR IN PLANT DEVELOPMENT

15 Fortuitously, it was discovered that the GUS cassette in ET199 described Section 7.2.2, above, is situated approximately 1 kb upstream from the SCR gene. The SCR cDNA was labelled and used to probe genomic DNA from WT and ET199 plants. The band pattern seen in the Southern was completely 20 consistent with a T-DNA inserted 1 kb upstream of the putative SCARECROW start site. Subsequently, a DNA fragment was PCR amplified using a primer within the T-DNA and a primer within SCARECROW. The size of this fragment was also consistent with the predicted insertion site. 25 sequencing of the PCR fragment confirmed the presence of SCARECROW sequence. Mutants in the SCR gene are completely lacking one of the radial layers between the epidermis and pericycle in both primary and lateral roots, due to the absence of specific cell division during embryogenesis and of 30 the cortex/endodermal initial during post-embryonic growth. The expression pattern (described in Section 7.2.2., above) that was observed in the central cells of the developing LRP of ET199 provide strong evidence that the cells in this

region are involved in the establishment of the meristematic 35 initials. More importantly, these results dem nstrate that transcriptional activation of the SCR g n plays a major role in the d velopment of the Arabidopsis LRP. Furthermore,

thes r sults d monstrate that a transg n can be expressed under the control of SCR gene transcriptional regulatory 1 m nts in a dev lopm ntal and tissue-sp cific manner.

8. EXAMPLE 3: ACTIVITY OF ARABIDOPSIS SCR PROMOTER IN TRANSGENIC ROOTS

been determined by analysis of an enhancer trap line, ET199, in which a GUS coding region with a minimal promoter was fortuitously inserted 1 kb upstream of the SCR coding region (see supra). In ET199 plants, GUS expression is detected in the endodermis, endodermal initials and sometimes in the quiescent center (QC) of the root. See supra and Malamy and Benfey, 1997, Dev. 124:33-44. This expression pattern of SCR in the primary root has been confirmed by in situ analysis (See supra and Di Laurenzio et al., 1996, Cell 86:423-433).

of 5' sequence upstream of the Arabidopsis SCR coding region is sufficient to confer SCR expression pattern to a heterologous gene. The 5' sequence used in these studies starts from the Hind III site approximately 2.5 kb upstream of the ATG initiation site and extends 3' downstream to the base pair immediately upstream of the ATG initiation site (see FIG. 14). This 5' sequence was fused to a GUS coding sequence. The resulting SCR promoter::GUS construct was incorporate into an Agrobacterium vector, which was used to transform and generate transgenic roots using standard procedures.

A large number of roots were regenerated. They
show GUS staining pattern that is similar to the SCR
expression pattern in ET199 plants (Figure 19, Panel f).
Since organs regenerated from callus often have an abnormal
morphology, transgenic roots were transferred to liquid
culture. Roots grown in liquid culture appeared
morphologically normal and showed GUS expression in the
ndod rmis, endodermal initial and QC (Figur 19, Pan 1 g),
similar to the xpression patt rn of SCR s en in th

enhancer trap line ET199. These results indicate that the 2.5 kb region upstream of the SCR start site is sufficient to confer the SCR expression patt rn in th root.

The expression of the SCR promoter::GUS construct 5 was also examined in scr mutant background. The scr mutant has an altered root organization (see, supra). Whereas the wild-type root of Arabidopsis has four distinct cell layers surrounding the vascular tissue, the roots of scr mutant have only three.

that contained a SCR promoter::GUS construct. As in the wild-type, a large number of transgenic roots were formed that had detectable GUS expression (Figure 20, Panel a). These roots were shorter than wild-type regenerated roots, 15 consistent with the shorter root phenotype of the scr mutant.

Additional transgenic root experiments demonstrated that the SCR gene under control of its own promoter can rescue the scr mutant phenotype. Transgenic scr roots were generated that contained the full length SCR gene

- 20 under the control of its own promoter. The length of transgenic roots containing the construct were longer than those of the scr mutant, indicating that the introduced SCR gene partially rescued the mutant. Whereas scr regenerated roots that carried the SCR promoter::GUS construct were very
- 25 short (Figure 21, Panel a; and Figure 20, Panel a), roots transformed with the SCR promoter and coding region were noticeably longer (Figure 21, Panel b). The difference was even more obvious in liquid culture, in which scr mutant roots remained short (Figure 21, Panel c), while SCR gene
- 30 complemented scr mutant roots were long and resembled wildtype roots (Figure 21, Panel d).

Anatomical studies of the regenerated roots confirmed the ability of the SCR promoter::SCR gene construct to rescue the scr mutant phenotype. Whereas regenerated 35 roots of scr mutant were missing an internal layer (Figure 21, Panel e), the scr mutant roots that wer transformed with the SCR promot r::SCR gene construct had a radial

organization that res mbled wild-type root (Figure 21, Pan 1 f).

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9. EXAMPLE 4: ISOLATION SCR SEQUENCES USING PCR-CLONING STRATEGY

Based on the comparison of the sequences of SCR paralogs in Arabidopsis, degenerate primers SCR3AII, SCR5AII and SCR5B were designed and used in PCR amplification of SCR sequences from genomic DNA of various plant species. amplification was performed according to condition described in Section 5.1.1., supra, using DNA isolated from maize plants grown from a commercial seed mixture. Amplification products (104 bp fragment for the SCR5B+SCR3AII primer combination; 146 bp fragment for the SCR5AII+SCR3AII primer combination) were obtained, and each cloned into a T/A vector (Invitrogen, San Diego, CA) and sequenced. Two of the three different types of clones obtained had deduced amino acid sequences that were very similar to a part of the Arabidopsis SCR protein (i.e., approximately 90% identity), suggesting that they represent parts from two different alleles of the maize SCR gene (i.e., ZCR gene). The two clones each had only two conservative changes in their nucleotide sequence.

subsequently used as a probe for screening of a genomic

library generated in lambda BlueSTAR vector (NOVAGEN) from maize (HiII line) genomic DNA. The screening was performed according to the standard procedures described in Genius
System User's Guide For Membrane Hybridization (Boehringer-Mannheim): The probe was a single-strand DNA molecule corresponding to the ZmScll fragment produced by PCR (Genius, Boehringer-Mannheim). Hybridization was performed according to recommendations of the manufacturer's manual (Boehringer-Mannheim). Prehybridization was for 2 hr in 50% formamide hybridization solution at 42°C. Hybridization was overnight at 42°C with 200 ng/ml probe concentration.

Filt rs were washed twic at room temperatur in 2xSSC, 0.1%

SDS for 5 min, and for string nt washing at 65°C in 0.5xSSC,0.1% SDS twice for 15 min.

A positive clone was identifi d. The clone contained a 13 kb insert, which was subcloned into a plasmid 5 vector. The resulting plasmid was designated pZCR. A 5 kb Eco RI fragment containing the maize SCR (ZCR) sequence was subcloned and sequenced. The nucleotide sequence of the region containing a partial ZCR coding sequence is shown in FIG. 17A and the corresponding deduced amino acid sequence is shown in FIG. 17B. The ZCR protein contain a segment that is highly homologous to a corresponding segment in the Arabidopsis SCR protein (FIG. 17B). This segment is flanked by segments of low homology. Thus, it is possible that the genomic clone of ZCR is a composite clone, containing 15 sequences that are not ZCR sequences.

The deduced ZCR protein sequence was aligned with that of Arabidopsis SCR protein. The comparison revealed new conserved sites in the SCR coding sequence which were used to design new, more specific PCR primers (i.e., 1F, 1R, and 4R) 20 for use in amplification of SCR sequences from yet other plant species.

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Using combinations of primers 1F+1R and 1F+4R,
PCR amplification were performed as described in section
5.1.1.. Two DNA of expected size were obtain from soybean:
25 a 247 bp DNA from the 1F+1R primer combination and a 379 bp
DNA from the 1F+4R primer combination. A DNA of expected
size (247 kb) was obtained from carrot and spruce when their
genomic DNA was amplified using 1F+4R primer combination.
The nucleotide sequences of the 379 kb soybean DNA (SRPg1),
30 the 247 kb DNA from carrot (SRPd1) and spruce (SRPp1) are
shown in FIGS. 16K-M. The corresponding deduced amino acid
sequences of these amplified sequences are shown in FIG. 18.
Comparison of these partial SCR coding sequences indicate
this approach isolated DNA sequences that encode SCR proteins
35 with amino acid sequences that are very similar but not

identical to a segment f Arabidopsis SCR protein (see FIG.

18).

10. EXAMPLE 5. EXPRESSION PATTERN OF MAIZE ZCR GENE IN ROOT TISSUE

Th s experiments examined the expr ssion patt rn of ZCR in the primary root and quiescent centers of maize The expression pattern was determined by in situ hybridization using a ZCR RNA probe, corresponding to an amino acid segment region that is highly homologous to a corresponding segment of the Arabidopsis SCR protein. experiment was carried out as follows. Restriction fragments containing the maize ZCR sequence were isolated from pZCR and subcloned into a pBluescript vector for in vitro transcription. The probe was synthesized using conditions described in the Genius Dig RNA labeling kit. The pBluescript plasmid was linearized, and 1 μ g was used as a template to synthesize digoxigenin-labeled RNA using the T7 polymerase. The RNA probe was subjected to mild alkali hydrolysis by heated at 60°C for 1 hr in 100 mM carbonate buffer (pH 10.2) to yield a probe size of approximately 0.15 Probe concentration for hybridization was optimized at 1 20 μg/ml/kb. In situ hybridization of root tips from 48 to 72 hr-old maize seedlings or excised quiescent centers (QCs) of roots were carried out following procedures described in Section 6.1.6., supra.

The results show that ZCR expression in maize

primary roots is localized to a file of cells that is
identified as the endodermal layer. The expression pattern
continues in a single uninterrupted file through the QC which
consists of approximately 1000-1500 cells (FIG. 22).

In two-week old regenerating QCs, ZCR expression is found in a file of cells extending through the newly formed apex. Thus, the regenerated roots exhibits a ZCR expression pattern that is similar to that seen in the primary root, even though the root apex does not contain the normal arrangement of cell files at this stage.

35 ZCR expression during regeneration of the root apex was als xamined. In the initial stages of regeneration, cell proliferation occurs to fill in the

removed tissue and begins to r generat the basic shap of the root tip. All cells on the blunt dge of the root appears to contribute to the new population of c lls. The ZCR expression pattern indicates that molecular signals are differentially present in these cells at an early stage in regeneration. The gene appears to be diagnostic of cells that are preparing to undergo asymmetrical division in order to re-establish the normal organization of the root apex from the large undifferentiated cells. The results indicate that 2CR expression is required for pattern formation since it is expressed prior to the generation of any specific anatomical pattern in the newly formed not tissue.

11. EXAMPLE 6. EXPRESSION PATTERN OF ZCR GENE IN SOYBEAN ROOTS AND ROOT NODULES

SCR expression in soybean roots and nodules was examined using in situ hybridization with a SCR probe. The procedure used were as described in Sections 6.1.6. and 11.

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In primary roots, SCR is expressed in the 20 endodermis. Expression was also found in cells at the root tip that are located at the distal end of the endodermal cell files. In soybean nodules, expression of SCR was detected in the peripheral tissue at the site of developing vascular strands. At later stages of vascular development within the 25 nodule, SCR expression was found flanking the vascular tissue. These results indicate that SCR is involved in regulating vascularization in the nodule by contributing to the radial organization that is required to generate endodermis. These findings indicate that SCR promoter may be 30 used to express proteins in a highly tissue-specific manner in soybean nodules. One application is to use SCR promoter to engineer nodules through production of components in a tissue-specific manner. Another application is that modification of the expression of SCR could enhance nodule 35 activity by improving vascularization and/or the number of endod rmal layers.

12. EXAMPLE 7. SCR EXPRESSION AFFECTS GRAVITROPISM OF AERIAL STRUCTURES

In addition t being defective in specific embryonic and postembryonic meristematic divisions, both the scr and the shr mutants have shoots that exhibit severely defective gravitropism. Complementation analysis showed that scr is allelic to a sgr (shoot gravitropism) mutant, sgr1. Four mutant alleles of SCR (i.e., scr1, scr2, sgr1-1 and sgr1-2) have been identified. All four of these mutants have normal root gravitropism and defective shoot gravitropism.

Etiolated hypocotyls of scr mutants placed on their sides do not respond to gravity even after 3 hr. Similar behaviors were observed with the inflorescence stems of sgr1-1 mutant, which do not curve upwards even after two days on their sides. In contrast, the roots of these plants respond rapidly to the change in orientation with the same kinetics as the wild type. Thus, mutations in the SCR gene lead to a radial pattern deficiency in the root but have no effect on root gravitropism.

Comparable results were also obtained for shr roots and for hypocotyls and inflorescence stems, i.e., data indicate that shr shows normal root gravitropism but almost no stem gravitropism.

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13. DEPOSIT OF MICROORGANISMS

The following microorganisms have been deposited in accordance with the terms of the Budapest Treaty with the American Type Culture Collection; 12301 Parklawn Drive, Rockville, MD 20852, U.S.A., on the dates indicated:

30	Microorganism	<u>Clone</u>	Accession	Date
	DH5a	pGEX-2TK' (pLIG 1-3/Sac+MOB1Sac	98031	April 26, 1996
	DH5a	pNYH1 (Zm-scllb)	98032	April 26, 1996
35	DH5a	pNYH2 (Zm-scll)	98033	April 26, 1996
	DH5a	pNYH3 (2m-scl2)	98034	April 26, 1996
	DH5a	pzcr		April 18, 1997

Although the inv ntion is described in detail with referenc to specific embodim nts th r of, it will be understood that variations which are functionally equivalent are within the scope of this invention. Indeed, various modifications of the invention in addition to those shown and described herein will become apparent to those skilled in the art from the foregoing description and accompanying drawings such modifications are intended to fall within the scope of the appended claims.

Various publications are cited herein, each of the disclosures of which is incorporated by reference in its entirety.

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International Application No: PCT/

MICROORGANISMS			
Optional Sheet in connection with the microorganism referred to on page 86, lines 25-37 of the description			
A. IDENTIFICATION OF DEPOSIT			
Further deposits are identified on an additional sheet '			
Name of depositary institution '			
American Type Culture Collection			
Address of depositery institution (including postal code and country)			
12301 Parklawn Drive			
Rockville, MD 20852 US			
Date of deposit * April 26, 1998 Accession Number * 98031			
B. ADDITIONAL INDICATIONS "(neave blank if not applicable). This information is continued on a separate attached sheet			
C. DESIGNATED STATES FOR WHICH INDICATIONS ARE MADE " No. 100 CO. 100			
O. DESIGNATES OFFICE VIOLENCE OF THE PROPERTY			
D. SEPARATE FURNISHING OF INDICATIONS (leave blank if not applicable)			
The Indications listed below will be submitted to the international Bureau lister* (Specify the general nature of the indications e.g., "Accession Number of Deposit")			
E. This sheet was received with the International application when filed (to be checked by the receiving Office)			
Vugina L. lily			
(Authorized Officer)			
☐ The date of receipt (from the applicant) by the International Bureau *			
was			
(Authorized Officer)			
Form PCT/RO/134 (January 1981)			

- 88 -

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Internati nal Application No: PCT/

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Form PCT/RO/134 (cont.)

American Type Culture Collection

12301 Parkiswn Drive Rockville, MD 20852 US

Accession No.	Date of Deposit		
98032	April 26, 1996		
98033	April 26, 1996		
98034	April 26, 1996		
	April 18, 1997		

PCT/US97/07022

WO 97/41152

SEQUENCE LISTING

(1) GENERAL INFORMATION:

- (i) APPLICANT: Benfey, Phillip N. Di Laurenzio, Laura Wysocka-Diller, Joanna Malamy, Jocelyn E. Pysh, Leonard Helaruitta, Yrjo
- (ii) TITLE OF INVENTION: SCARECROW GENE, PROMOTER AND USES
- (iii) NUMBER OF SEQUENCES: 67
 - (iv) CORRESPONDENCE ADDRESS:

 - (A) ADDRESSEE: Pennie & Edmonds LLP (B) STREET: 1155 Avenue of the Americas
 - (C) CITY: New York
 - (D) STATE: New York

 - (E) COUNTRY: USA (F) ZIP: 10036-2711
 - (v) COMPUTER READABLE FORM:
 - (A) MEDIUM TYPE: Floppy disk
 - (B) COMPUTER: IBM PC compatible
 - (C) OPERATING SYSTEM: PC-DOS/MS-DOS
 - (D) SOFTWARE: PatentIn Release #1.0, Version #1.30
 - (vi) CURRENT APPLICATION DATA:
 - (A) APPLICATION NUMBER: US
 - (B) FILING DATE:
 - (C) CLASSIFICATION:
- (vii) PRIOR APPLICATION DATA:
 - (A) APPLICATION NUMBER: US 08/638,617
 - (B) FILING DATE: 26-APR-1996
- (viii) ATTORNEY/AGENT INFORMATION:
 - (A) NAME: Coruzzi, Laura A.
 - (B) REGISTRATION NUMBER: 30,742
 - (C) REFERENCE/DOCKET NUMBER: 005914-0056-999
 - (ix) TELECOMMUNICATION INFORMATION:
 - (A) TELEPHONE: (212) 790-9090
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 - (C) TELEX: 66141 PENNIE
- (2) INFORMATION FOR SEQ ID NO:1:
 - (i) SEQUENCE CHARACTERISTICS:
 - (A) LENGTH: 2163 base pairs
 - (B) TYPE: nucleic acid
 - (C) STRANDEDNESS: unknown
 - (D) TOPOLOGY: unknown
 - (ii) MOLECULE TYPE: DNA (genomic)
 - (xi) SEQUENCE DESCRIPTI N: SEQ ID NO:1:
- CCTTATTTAT AACCATGCAA TCTCACGACC AACAACCCTT CAATCTCCAT GGCGGAATCC

GGCGATTTCA ACGGTGGTCA ACCTCCTCCT CATAGTCCTC TGAGAACAAC TTCTTCCGGT	120
AGTAGCAGCA GCAACAACCG TGGTCCTCCT CCTCCTCCTC CTCCTCCTTT AGTGATGGTG	180
AGAAAAAGAT TAGCTTCCGA GATGTCTTCT AACCCTGACT ACAACAACTC CTCTCGTCCT	240
CCTCGCCGTG TCTCTCACCT TCTTGACTCC AACTACAATA CTGTCACACC ACAACAACCA	300
CCGTCTCTTA CGGCGGCGC TACTGTATCT TCTCAACCAA ACCCACCACT CTCTGTTTGT	360
GGCTTCTCTG GTCTTCCCGT TTTTCCTTCA GACCGTGGTG GTCGGAATGT TATGATGTCC	420
GTACARCCAR TGGATCARGA CTCTTCATCT TCTTCTGCTT CACCTACTGT ATGGGTTGAC	480
GCCATTATCA GAGACCTTAT CCATTCCTCA ACTTATCCAA	540
ARCETTAGAG ACATTATCTT CCCTTGTAAC CCAAATCTCG GTGCTCTTCT TGAATACAGG	600
CTCCGATCTC TCATGCTCCT TGATCCTTCC TCTTCCTCTC ACCCTTCTCC TCAAACTTTC	660
GRACCTCTCT ATCAGATCTC CAACAATCCT TCTCCTCCAC AACAGCAACA GCAGCACCAA	720
CANCANCARC ANCAGCATAN GCCTCCTCCT CCTCCGATTC AGCAGCANGA ANGAGANANT	780
TCTTCTACCG ATGCACCACC GCAACCAGAG ACAGTGACGG CCACTGTTCC CGCCGTCCAA	840
ACAAATACGG CGGAGGCTTT AAGAGAGAGG AAGGAAGAGA TTAAGAGGCA GAAGCAAGAC	900
GAAGAAGGAT TACACCTTCT CACATTGCTG CTACAGTGTG CTGAAGCTGT CTCTGCTGAT	960
AATCTCGAAG AAGCAAACAA GCTTCTTCTT GAGATCTCTC AGTTATCAAC TCCTTACGGG	1020
ACCTCAGCGC AGAGAGTAGC TGCTTACTTC TCGGAAGCTA TGTCAGCGAG ATTACTCAAC	1080
TCGTGTCTCG GAATTTACGC GGCTTTGCCT TCACGGTGGA TGCCTCAAAC GCATAGCTTG	1140
AAAATGGTCT CTGCGTTTCA GGTCTTTAAT GGGATAAGCC CTTTAGTGAA ATTCTCACAC	1200
TTTACAGCGA ATCAGGCGAT TCAAGAAGCA TTTGAGAAAG AAGACAGTGT ACACATCATT	1260
GACTTGGACA TCATGCAGGG ACTTCAATGG CCTGGTTTAT TCCACATTCT TGCTTCTAGA	1320
CCTGGAGGAC CTCCACACGT GCGACTCACG GGACTTGGTA CTTCCATGGA AGCTCTTCAG	1380
GCTACAGGGA AACGTCTTTC GGATTTCACA GATAAGCTTG GCCTGCCTTT TGAGTTCTGC	1440
CCTTTAGCTG AGAAAGTTGG AAACTTGGAC ACTGAGAGAC TCAATGTGAG GAAAAGGGAA	1500
GCTGTGGCTG TTCACTGGCT TCAACATTCT CTTTATGATG TCACTGGCTC TGATGCACAC	1560
ACTOTOTGGT TACTOCARAG GTARRATARA CATTACOTTT TARTCACTCT TTATCTATAR	1620
ATTATTTAA GATTATATAG GARAGATATG TTCTAAAAAG CTGGCTTTTT TGGTTAATGA	1680
TTGGGGAATG AACAGATTAG CTCCTAAAGT TGTGACAGTA GTGGAGCAAG ATTTGAGCCA	1740
CGCTGGTTCT TTCTTAGGAA GATTTGTAGA GGCAATACAT TACTACTCTG CACTCTTTGA	1800
CTCACTGGGA GCAAGCTACG GCGAAGAGAG TGAAGAGAGA CATGTCGTGG AACAGCAGCT	1860
ATTATCGARA GAGATACGGA ATGTATTAGC GGTTGGAGGA CCATCGAGAA GCGGTGAAGT	1920
GAAGTTTGAG AGCTGGAGGG AGAAATTGCA ACAATGTGGG TTTAAAGGTA TATCTTTAGC	1980
TGGAAATGCA GCTACACAAG CGACTCTACT GTTGGGAATG TTTCCTTCGG ATGGTTACAC	2040
TTTGGTTGAT GATAATGGTA CACTTAAGCT TGGATGGAAA GATCTTTCGT TACTCACTGC	2100

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TTCAGCTTGG ACCCCCGTT CTTAGTTTTC TTCTCCTTTT TCACAAACAA TGTGCCCATA 2160 2163

(2) INFORMATION FOR SEQ ID N :2:

- (i) SEQUENCE CHARACTERISTICS:
 - (A) LENGTH: 653 amino acide
 - (B) TYPE: amino acid
 - (C) STRANDEDNESS: unknown (D) TOPOLOGY: unknown

(ii) MOLECULE TYPE: protein

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:2:

245

Het Ala Glu Ser Gly Asp Phe Asn Gly Gly Gln Pro Pro Pro His Ser Pro Leu Arg Thr Thr Ser Ser Gly Ser Ser Ser Asn Asn Arg Gly Pro Pro Pro Pro Pro Pro Pro Leu Val Het Val Arg Lys Arg Leu Ala Ser Glu Met Ser Ser Asn Pro Asp Tyr Asn Asn Ser Ser Arg Pro Pro Arg Arg Val Ser His Leu Leu Asp Ser Asn Tyr Asn Thr Val Thr Pro Gin Gin Pro Pro Ser Leu Thr Ala Ala Ala Thr Val Ser Ser Gin Pro Asn Pro Pro Leu Ser Val Cys Gly Phe Ser Gly Leu Pro Val Phe Pro Ser Asp Arg Gly Gly Arg Asn Val Met Met Ser Val Gln Pro Met Asp Gln Asp Ser Ser Ser Ser Ser Ala Ser Pro Thr Val Trp Val Asp 135 Ala Ile Ile Arg Asp Leu Ile His Ser Ser Thr Ser Val Ser Ile Pro Gin Leu Ile Gin Asn Val Arg Asp Ile Ile Phe Pro Cys Asn Pro Asn Leu Gly Ala Leu Leu Glu Tyr Arg Leu Arg Ser Leu Met Leu Leu Asp 185 Pro Ser Ser Ser Ser Asp Pro Ser Pro Gln Thr Phe Glu Pro Leu Tyr Gin Ile Ser Asn Asn Pro Ser Pro Pro Gin Gin Gin Gin His Gin Gln Gln Gln Gln His Lys Pro Pro Pro Pro Pro Ile Gln Gln 235 Glu Arg Glu Asn Ser Ser Thr Asp Ala Pro Pr In Pro Glu Thr Val

250

Thr Ala Thr Val Pro Ala Val Gln Thr Asn Thr Ala Glu Ala Leu Arg lu Arg Lys Glu lu Ile Lys Arg Gln Lys Gln Asp Glu Glu Gly Leu His Leu Leu Thr Leu Leu Gln Cys Ala Glu Ala Val Ser Ala Asp Asn Leu Glu Glu Ala Asn Lys Leu Leu Leu Glu Ile Ser Gln Leu Ser 305 Thr Pro Tyr Gly Thr Ser Ala Gln Arg Val Ala Ala Tyr Phe Ser Glu Ala Met Ser Ala Arg Leu Leu Asn Ser Cys Leu Gly Ile Tyr Ala Ala 345 Leu Pro Ser Arg Trp Met Pro Gln Thr His Ser Leu Lys Met Val Ser Ala Phe Gln Val Phe Asn Gly Ile Ser Pro Leu Val Lys Phe Ser His 370 380 Phe Thr Ala Asn Gln Ala Ile Gln Glu Ala Phe Glu Lys Glu Asp Ser 390 Val His Ile Ile Asp Leu Asp Ile Met Gln Gly Leu Gln Trp Pro Gly 405 Leu Phe His Ile Leu Ala Ser Arg Pro Gly Gly Pro Pro His Val Arg Leu Thr Gly Leu Gly Thr Ser Met Glu Ala Leu Gln Ala Thr Gly Lys 440 Arg Leu Ser Asp Phe Thr Asp Lys Leu Gly Leu Pro Phe Glu Phe Cys 455 Pro Leu Ala Glu Lys Val Gly Asn Leu Asp Thr Glu Arg Leu Asn Val 470 Arg Lys Arg Glu Ala Val Ala Val His Trp Leu Gln His Ser Leu Tyr 490 Asp Val Thr Gly Ser Asp Ala His Thr Leu Trp Leu Leu Gln Arg Leu 500 Ala Pro Lys Val Val Thr Val Val Glu Gln Asp Leu Ser His Ala Gly 520 Ser Phe Leu Gly Arg Phe Val Glu Ala Ile His Tyr Tyr Ser Ala Leu 535 Phe Asp Ser Leu Gly Ala Ser Tyr Gly Glu Glu Ser Glu Glu Arg His 550 Val Val Glu Gln Gln Leu Leu Ser Lys Glu Ile Arg Asn Val Leu Ala 570 Val Gly Gly Pro Ser Arg Ser Gly Glu Val Lys Phe Glu Ser Trp Arg **585** Glu Lys Met Gln Gln Cys Gly Ph Lys Gly Ile Ser Leu Ala Gly Asn Ala Ala Thr Gln Ala Thr Leu Leu Cly Met Phe Pro Ser Amp Gly

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> 615 620 610

Tyr Thr Leu Val Asp Asp Asn Gly Thr Leu Lys Leu Gly Trp Lys Asp 635 625 630

Leu Ser Leu Leu Thr Ala Ser Ala Trp Thr Pro Arg Ser

- (2) INFORMATION FOR SEQ ID NO:3:
 - (1) SEQUENCE CHARACTERISTICS:
 - (A) LENGTH: 23 amino acids
 - (B) TYPE: amino acid
 - (C) STRANDEDNESS: unknown
 - (D) TOPOLOGY: unknown
 - (ii) MOLECULE TYPE: peptide
 - (x1) SEQUENCE DESCRIPTION: SEQ ID NO: 3:

Pro Ala Val Gln Thr Asn Thr Ala Glu Ala Leu Arg Glu Arg Lys Glu

Glu Ile Lys Arg Gln Lys Gln

- (2) INFORMATION FOR SEQ ID NO:4:
 - (i) SEQUENCE CHARACTERISTICS:
 - (A) LENGTH: 23 amino acids
 - (B) TYPE: amino acid
 - (C) STRANDEDNESS: unknown
 - (D) TOPOLOGY: unknown
 - (ii) MOLECULE TYPE: peptide
 - (xi) SEQUENCE DESCRIPTION: SEQ ID NO:4:

Leu Lys Arg Ala Arg Asn Thr Glu Ala Ala Arg Arg Ser Arg Ala Arg

Lys Leu Gln Arg Met Lys Gln 20

- (2) INFORMATION FOR SEQ ID NO:5:
 - (i) SEQUENCE CHARACTERISTICS:
 - (A) LENGTH: 23 amino acids (B) TYPE: amino acid (C) STRANDEDNESS: unknown

 - (D) TOPOLOGY: unknown
 - (ii) MOLECULE TYPE: peptide
 - (xi) SEQUENCE DESCRIPTION: SEQ ID NO:5:

Arg Arg Leu Ala Gln Asn Arg Glu Ala Ala Arg Lys Ser Arg Leu Arg 10

Lys Lys Ala Tyr Val Gln Gln

- (2) INFORMATI N FOR SEQ ID NO:6:
 - (1) SEQUENCE CHARACTERISTICS:
 - (A) LENGTH: 23 amino acids
 - (B) TYPE: amino acid
 - (C) STRANDEDNESS: unknown
 - (D) TOPOLOGY: unknown
 - (ii) MOLECULE TYPE: peptide
 - (xi) SEQUENCE DESCRIPTION: SEQ ID NO:6:

Ile Arg Arg Glu Arg Asn Lys Met Ala Ala Ala Lys Cys Arg Asn Arg

Arg Arg Glu Leu Thr Asp Thr 20

- (2) INFORMATION FOR SEQ ID NO:7:
 - (i) SEQUENCE CHARACTERISTICS:
 - (A) LENGTH: 23 amino acids

 - (B) TYPE: amino acid (C) STRANDEDNESS: unknown
 - (D) TOPOLOGY: unknown
 - (ii) MOLECULE TYPE: peptide
 - (xi) SEQUENCE DESCRIPTION: SEQ ID NO:7:

Arg Lys Arg Met Arg Asn Arg Ile Ala Ala Ser Lys Cys Arg Lys Arg

Lys Leu Glu Arg Ile Ala Arg

- (2) INFORMATION FOR SEQ ID NO:8:
 - (1) SEQUENCE CHARACTERISTICS:
 - (A) LENGTH: 23 amino acids
 - (B) TYPE: amino acid
 - (C) STRANDEDNESS: unknown
 - (D) TOPOLOGY: unknown
 - (ii) MOLECULE TYPE: peptide
 - (xi) SEQUENCE DESCRIPTION: SEQ ID NO:8:

Val Arg Leu Met Lys Asn Arg Glu Ala Ala Arg Glu Cys Arg Arg Lys

Lys Lys lu Tyr Val Lys Cys

(2) INFORMATI N FOR SEQ ID NO:9:

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- (i) SEQUENCE CHARACTERISTICS:
 - (A) LENGTH: 23 amino acids (B) TYPE: amin acid (C) STRANDEDNESS: unknown

 - (D) TOPOLOGY: unknown
- (ii) MOLECULE TYPE: peptide
- (xi) SEQUENCE DESCRIPTION: SEQ ID NO:9:

Lys Arg Lys Glu Ser Asn Arg Glu Ser Ala Arg Arg Ser Arg Tyr Arg

Lys Ala Ala His Leu Lys Glu 20

- (2) INFORMATION FOR SEQ ID NO:10:
 - (i) SEQUENCE CHARACTERISTICS:
 - (A) LENGTH: 23 amino acids
 - (B) TYPE: amino acid
 - (C) STRANDEDNESS: unknown (D) TOPOLOGY: unknown
 - (ii) MOLECULE TYPE: peptide
 - (xi) SEQUENCE DESCRIPTION: SEQ ID NO:10:

Het Arg Gln Ile Arg Asn Arg Asp Ser Ala Het Lys Ser Arg Glu Arg 10

Lys Lys Ser Tyr Ile Lys Asp

- (2) INFORMATION FOR SEQ ID NO:11:
 - (i) SEQUENCE CHARACTERISTICS:
 - (A) LENGTH: 23 amino acids
 - (B) TYPE: amino acid
 - (C) STRANDEDNESS: unknown
 - (D) TOPOLOGY: unknown
 - (ii) MOLECULE TYPE: peptide
 - (xi) SEQUENCE DESCRIPTION: SEQ ID NO:11:

Arg Arg Met Val Ser Asn Arg Glu Ser Ala Arg Arg Ser Arg Lys Lys

Lys Gln Ala His Leu Ala Asp

- (2) INFORMATION FOR SEQ ID NO:12:
 - (1) SEQUENCE CHARACTERISTICS:
 - (A) LEN TH: 43 amino acids (B) TYPE: amino acid (C) STRANDEDNESS: unknown

- (D) TOPOLOGY: unknown
- (ii) MOLECULE TYPE: peptide
- (xi) SEQUENCE DESCRIPTION: SEQ ID NO:12:
- Ala Phe Glu Lys Glu Asp Ser Val His Ile Ile Asp Leu Asp Ile Met
- Gin Gly Leu Gin Trp Pro Gly Leu Phe His Ile Leu Ala Ser Arg Pro
- Gly Gly Pro Pro His Val Arg Leu Thr Gly Leu
- (2) INFORMATION FOR SEQ ID NO:13:
 - (i) SEQUENCE CHARACTERISTICS:
 - (A) LENGTH: 43 amino acids
 - (B) TYPE: amino acid
 - (C) STRANDEDNESS: unknown
 - (D) TOPOLOGY: unknown
 - (ii) MOLECULE TYPE: peptide
 - (xi) SEQUENCE DESCRIPTION: SEQ ID NO:13:
 - Ala Val Lys Asn Glu Ser Phe Val His Ile Ile Asp Phe Gln Ile Ser
 - Gin Gly Gly Gin Trp Val Ser Leu Ile Arg Ala Leu Gly Ala Arg Pro
 - Gly Gly Pro Pro Asn Val Arg Ile Thr Gly Ile
- (2) INFORMATION FOR SEQ ID NO:14:
 - (i) SEQUENCE CHARACTERISTICS:
 - (A) LENGTH: 43 amino acids (B) TYPE: amino acid

 - (C) STRANDEDNESS: unknown
 - (D) TOPOLOGY: unknown
 - (ii) MOLECULE TYPE: peptide
 - (xi) SEQUENCE DESCRIPTION: SEQ ID NO:14:
 - Ala Met Glu Gly Glu Lys Met Val His Val Ile Asp Leu Asp Ala Ser
 - Glu Pro Ala Gln Trp Leu Ala Leu Leu Gln Ala Phe Asn Ser Arg Pro
 - Glu Gly Pro Pr His Leu Arg Ile Thr Gly Val 35
- (2) INFORMATION FOR SEQ ID NO:15:

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- (i) SEQUENCE CHARACTERISTICS:
 - (A) LENGTH: 29 amino acids
 - (B) TYPE: amino acid
 - (C) STRANDEDNESS: unknown
 - (D) TOPOLOGY: unknown
- (ii) MOLECULE TYPE: peptide
- (xi) SEQUENCE DESCRIPTION: SEQ ID NO:15:

Ala Ile Lys Gly Glu Glu Val His Ile Ile Asp Phe Asp Ile Asn

Gln Gly Asn Gln Tyr Met Thr Leu Ile Arg Ser Ile Ala

- (2) INFORMATION FOR SEQ ID NO:16:
 - (i) SEQUENCE CHARACTERISTICS:
 - (A) LENGTH: 26 amino acids
 - (B) TYPE: amino acid
 - (C) STRANDEDNESS: unknown
 - (D) TOPOLOGY: unknown
 - (ii) MOLECULE TYPE: peptide
 - (xi) SEQUENCE DESCRIPTION: SEQ ID NO:16:

Ile His Val Ile Asp Phe Xaa Leu Gly Val Gly Gln Trp Ala Ser

Phe Leu Gln Glu Leu Ala His Arg Arg Gly

- (2) INFORMATION FOR SEQ ID NO:17:
 - (i) SEQUENCE CHARACTERISTICS:
 - (A) LENGTH: 36 amino acids

 - (B) TYPE: amino acid (C) STRANDEDNESS: unknown
 - (D) TOPOLOGY: unknown
 - (ii) MOLECULE TYPE: peptide
 - (xi) SEQUENCE DESCRIPTION: SEQ ID NO:17:

Val His Ile Ile Xaa Phe Xaa Leu Met Gln Gly Leu Gln Trp Pro Ala

Leu Het Asp Val Phe Ser Ala Arg Lys Gly Gly Pr Pro Lys Leu Arg

Ile Thr Gly Ile 35

- (2) INFORMATION FOR SEQ ID NO:18:
 - (1) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 1085 base pairs (B) TYPE: nucleic a id (C) STRANDEDNESS: unknown

- (D) TOPOLOGY: unknown

(ii) MOLECULE TYPE: cDNA

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:18:

GGCACGAGC	CAACGGGTCC	TGAGCTTCTT	C ACTTATATEC	ATATCTTGTA	TGAAGCCTGC	60
CCTTATTTC	AATTOGGTTA	TGAATCTGC1	AATGGAGCTA	TAGCTGAAGC	TGTGAAGAAC	120
GAAAGTTTT	TGCACATTAT	CGATTTCCAG	ATTTCTCAAG	GTGGTCAATG	GGTGAGTTTG	180
ATCCGTGCT	TTGGTGCTAG	ACCTGGTGGA	CCTCCGAACG	TTAGGATAAC	GGGAATTGAT	240
GATCCGAGA1	CATCGTTTGC	TCGTCAAGGA	GGACTTGAGT	TAGTTGGACA	AAGACTTGGG	300
AAGCTAGCT	AAATGTGCGG	TGTTCCGTTT	GAGTTCCATG	GAGCTGCTTT	ATGCTGCACG	360
GAAGTCGAAA	TCGAGAAGCT	aggagttaga	AATGGAGAAG	CCCTCCCCGT	TAACTTCCCG	420
CTTGTTCTTC	ACCACATGCC	TGATGAGAGT	GTAACTGTGG	AGAATCACAG	AGATAGATTG	480
TTGAGATTGG	TCAAACACTT	GTCACCAAAC	GTTGTGACTC	TGGTTGAGCA	AGAAGCGAAT	540
ACAAACACTG	CCCCGTTTCT	TCCCCGGTTT	GTCGAGACAA	TGAACCATTA	CTTGGCAGTT	600
TTCGAATCAA	TAGATGTGAA	ACTCGCTAGA	GATCACAAGG	AAAGGATCAA	TGTTGAGCAG	660
CATTGTTTGG	CTAGAGAGGT	TGTGAATCTT	ATAGCTTGTG	AAGGTGTTGA	AAGAGAAGAG	720
AGGCACGAGC	CACTAGGGAA	ATGGAGGTCT	CGGTTTCACA	TGGCGGGATT	TAAACCGTAT	780
CCTTTGAGCT	CGTATGTGAA	CGCAACAATC	AAAGGATTGC	TTGAGAGTTA	TTCAGAGAAG	840
TATACACTTG	AAGAAAGAGA	TGGAGCATTG	TATTTAGGAT	GGAAGAATCA	ACCTCTTATC	900
ACTTCTTGTG	CTTGGAGGTA	actaataaaa	ACCTTGTTCG	GTTTCAGAAG	AGATTAGAAA	960
CTTCTTTTAA	AGTTTGCAGA	ATCTGTTTGT	AAAAGTAAAA	CTCATGCATG	ATCCGNAGGA	1020
	AAATGTTGTA					1080
እእእእ						
						1085

(2) INFORMATION FOR SEQ ID NO:19:

- (1) SEQUENCE CHARACTERISTICS:
 - (A) LENGTH: 306 amino acids (B) TYPE: amino acid

 - (C) STRANDEDNESS: unknown (D) TOPOLOGY: unknown
- (ii) MOLECULE TYPE: protein
- (xi) SEQUENCE DESCRIPTION: SEQ ID NO:19:
- Gly Thr Ser Pr Thr Gly Pro lu Leu Leu Thr Tyr Het His Ile Leu 10 15

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lu Ala Cys Pr Tyr Ph Lys Phe Gly Tyr Glu Ser Ala Asn Gly 20 25 30 Ala Ile Ala Glu Ala Val Lys Asn Glu Ser Phe Val His Ile Ile Asp Phe Gin Ile Ser Gin Gly Gly Gin Trp Val Ser Leu Ile Arg Ala Leu Gly Ala Arg Pro Gly Gly Pro Pro Asn Val Arg Ile Thr Gly Ile Asp 65 70 75 80 Asp Pro Arg Ser Ser Phe Ala Arg Gln Gly Gly Leu Glu Leu Val Gly 85 90 95 Gln Arg Leu Gly Lys Leu Ala Glu Het Cys Gly Val Pro Phe Glu Phe His Gly Ala Ala Leu Phe Cys Thr Glu Val Glu Ile Glu Lys Leu Gly Val Arg Asn Gly Glu Ala Leu Ala Val Asn Phe Pro Leu Val Leu His 130 135 140 His Met Pro Asp Glu Ser Val Thr Val Glu Asn His Arg Asp Arg Leu Leu Arg Leu Val Lys His Leu Ser Pro Asn Val Val Thr Leu Val Glu Gin Glu Ala Asn Thr Asn Thr Ala Pro Phe Leu Pro Arg Phe Val Glu Thr Met Asn His Tyr Leu Ala Val Phe Glu Ser Ile Asp Val Lys Leu Ala Arg Asp His Lys Glu Arg Ile Asn Val Glu Gln His Cys Leu Ala Arg Glu Val Glu Asn Leu Ile Ala Cys Glu Gly Val Glu Arg Glu Glu 225 230 240 Arg His Glu Pro Leu Gly Lys Trp Arg Ser Arg Phe His Met Ala Gly Phe Lys Pro Tyr Pro Leu Ser Ser Tyr Val Asn Ala Thr Ile Lys Gly 260 265 270 Leu Leu Glu Ser Tyr Ser Glu Lys Tyr Thr Leu Glu Glu Arg Asp Gly Ala Leu Tyr Leu Gly Trp Lys Asn Gln Pro Leu Ile Thr Ser Cys Ala 290 295 300 Trp Arg

(2) INFORMATION FOR SEQ ID NO:20:

- (i) SEQUENCE CHARACTERISTICS:
 - (A) LENGTH: 1231 base pairs

 - (B) TYPE: nucleic acid (C) STRANDEDNESS: unknown
 - (D) TOPOLOGY: unknown
- (11) MOLECULE TYPE: CDNA

(x1) SEQUENCE DESCRIPTION: SEQ ID NO:20:

GCTATGGAAG	GAGAGAAGAT	GGTTCATGTG	ATTGATCTCG	ATGCTTCTGA	GCCAGCTCAA	60
TGGCTTGCTT	TGCTTCAAGC	TTTTAACTCT	AGGCCTGAAG	GTCCACCTCA	TTTGAGAATC	120
ACTGGTGTTC	ATCACCAGAA	GGAAGTGCTT	GAACAAATGG	CTCATAGACT	CATTGAGGAA	180
GCAGAGAAAC	TCGATATCCC	GTTTCAGTTT	AATCCCGTTG	TGAGTAGGTT	AGACTGTTTA	240
aatgtagaac	AGTTGCGGGT	TAAAACAGGA	GAGGCCTTAG	CCGTTAGCTC	GGTTCTTCAA	300
TTGCATACCT	TCTTGGCCTC	TGATGATGAT	CTCATGAGAA	AGAACTGCGC	TTTACGGTTT	360
CAGAACAACC	CTAGTGGAGT	TGACTTGCAG	AGAGTTCTAA	TGATGAGCCA	TGGCTCTGCA	420
GCTGAGGCAC	GTGAGAATGA	TATGAGTAAC	AACAATGGGT	ATAGCCCTAG	CGGTGACTCG	480
GCCTCATCTT	TGCCTTTACC	AAGTTCAGGA	AGGACTGATA	GCTTCCTCAA	TGCTATTTGG	540
GGTTTGTCTC	CAAAGGTCAT	GGTGGTCACT	GAGCAAGACT	CAGACCACAA	CGGCTCCACA	600
CTAATGGAGA	GGCTATTAGA	ATCACTTTAC	ACCTACGCAG	CATTGTTTGA	TTGCTTGGAA	660
ACAAAAGTTC	CAAGAACGTC	TCAAGATAGG	ATCAAAGTGG	AGAAGATGCT	CTTCGGGGAG	720
Gagatcaaga	ACATCATATC	CTGCGAGGGA	TTTGAGAGAA	GAGAAAGACA	CGAGAAGCTT	780
GAGAAATGGA	GCCAGAGGAT	CGATTTGGCT	GGTTTTGGGA	ATGTTCCTCT	TAGCTATTAT	840
GCGATGTTGC	AGGCTAGGAG	ATTGCTTCAA	GGGTGCGGTT	TTGATGGGTA	TAGAATCAAG	900
GAAGAGAGCG	GGTGCGCAGT	AATTTGCTGG	CAAGATCGAC	CTCTATACTC	GGTATCAGCT	960
rggagatgca	GGAAGTGAAT	GATATATTAC	AGTTTGTCTT	CTATTTTGGT	TATGAGCAGA	1020
STCCCTTTCT	TTTTTGTATA	CATGGGGACA	CAATCTTAGT	TGTTTTGTGA	TGGTGACTTT	1080
CTGTCTCTTT	ATGCTATTTT	GGCTTAAATG	CTTCTACTGC	CTCTGCATGT	AAAGCCTTTG	1140
CTGTTGGTT	CAATTTGGTC	TGGTGTGGGT	GTAATACCAA	ACCARATCCA	ATTTGAGCTG	1200
LAGATAACTA	ATTTGATGAT	CGGCTCGTGC	C			1231

(2) INFORMATION FOR SEQ ID NO:21:

- (1) SEQUENCE CHARACTERISTICS:
 - (A) LENGTH: 325 amino acids
 (B) TYPE: amino acid
 (C) STRANDEDNESS: unknown
 (D) TOPOLOGY: unknown

(ii) MOLECULE TYPE: protein

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:21:

Ala Met Glu Gly Glu Lys Met Val His Val Ile Asp Leu Asp Ala Ser

lu Pro Ala Gln Trp Leu Ala Leu Leu Gln Ala Phe Asn Ser Arg Pr 20

lu Gly Pro Pro His Leu Arg Ile Thr Gly Val His His Gln Lys Glu 40 Val Leu Glu Gln Met Ala His Arg Leu Ile Glu lu Ala Glu Lys Leu Asp Ile Pro Phe Gln Phe Asn Pro Val Val Ser Arg Leu Asp Cys Leu Asn Val Glu Gln Leu Arg Val Lys Thr Gly Glu Ala Leu Ala Val Ser 85 90 95 Ser Val Leu Gln Leu His Thr Phe Leu Ala Ser Asp Asp Leu Het Arg Lys Asn Cys Ala Leu Arg Phe His Asn Asn Pro Ser Gly Val Asp Leu Gln Arg Val Leu Met Met Ser His Gly Ser Ala Ala Glu Ala Arg Glu Asn Asp Met Ser Asn Asn Asn Gly Tyr Ser Pro Ser Gly Asp Ser Ala Ser Ser Leu Pro Leu Pro Ser Ser Gly Arg Thr Asp Ser Phe Leu Asn Ala Ile Trp Gly Leu Ser Pro Lys Val Het Val Val Thr Glu Gln Asp Ser Asp His Asn Gly Ser Thr Leu Met Glu Arg Leu Leu Glu Ser Leu Tyr Thr Tyr Ala Ala Leu Phe Asp Cys Leu Glu Thr Lys Val Pro Arg Thr Ser Gin Asp Arg Ile Lys Val Glu Lys Met Leu Phe Gly Glu Glu Ile Lys Asn Ile Ile Ser Cys Glu Gly Phe Glu Arg Arg Glu Arg His Glu Lys Leu Glu Lys Trp Ser Gln Arg Ile Asp Leu Ala Gly Phe Gly Asn Val Pro Leu Ser Tyr Tyr Ala Met Leu Gln Ala Arg Arg Leu 275 280 285 Leu Gln Gly Cys Gly Phe Asp Gly Tyr Arg Ile Lys Glu Glu Ser Gly 290 295 300 Cys Ala Val Ile Cys Trp Gln Asp Arg Pro Leu Tyr Ser Val Ser Ala

Trp Arg Cys Arg Lys 325

(2) INFORMATION FOR SEQ ID NO:22:

- (i) SEQUENCE CHARACTERISTICS:
 - (A) LENGTH: 1368 base pairs
 - (B) TYPE: nucleic acid
 - (C) STRANDEDNESS: unknown
 - (D) TOPOLOGY: unknown
- (11) MOLECULE TYPE: CDNA

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 22:

•			•			
CTTTGTCAAT	GGTAAATGAG	CTGAGGCAGI	TAGTTTCTA1	CCAAGGAGAG	CCTTCTCAGA	60
GAATCGCAGC	TTACATGGTG	GAAGGTCTAG	CTGCAAGAAT	GCCGCTTC	GGAAAATTCA	120
TCTACAGAGC	ATTGAAATG	AAAGAGCCTC	CTTCGGATGA	GAGGCTTGC	GCTATGCAAG	180
TCCTGTTTGA	AGTCTGCCCT	TGTTTCAAGT	TCGGGTTTTT	AGCAGCTAAT	GGTGCGATAC	240
TTGAAGCAAT	CAAAGGTGAA	GAAGAAGTTO	: ACATAATCGA	TTTCGATATA	AACCAAGGA	300
ACCAATACAT	GACACTGATA	CGAAGCATTG	CTGAGTTGCC	TGGTAAACGA	CCTCGCCTGA	360
GGTTAACAGG	AATTGATGAC	CCTGAATCAG	TCCAACGCTC	CATTGGAGGG	CTAAGAATCA	420
TCGGTCTAAG	ACTCGAGCAA	CTCGCAGAGG	ATAATGGAGT	ATCCTTCAAA	TTCAAAGCAA	480
TGCCTTCAAA	GACTTCGATT	GTCTCTCCAT	CAACACTCGG	TTGCAAACCA	GGAGAAACCT	540
TAATAGTGAA	CTTTGCATTC	CAACTTCACC	ACATGCCTGA	CGAGAGTGTC	ACAACAGTAA	600
ACCAGCGGGA	CGAGCTACTT	CACATGGTCA	AAAGCTTAAA	CCCAAAGCTT	GTCACGGTCG	660
TTGAACAAGA	CGTGAACACA	AACACTTCAC	CGTTCTTTCC	CAGATTCATA	GAGGCTTACG	720
AATACTACTC	AGCAGTTTTC	GAGTCTCTAG	ACATGACACT	TCCAAGAGAA	AGCCAAGAGA	780
GGATGAATGT	AGAAAGACAG	TGTCTCGCTA	GAGACATAGT	CARCATTGTT	GCTTGCGAAG	840
GAGAAGAACG	Gatagagaga	TACGAGGCTG	CGGGAAAATG	GAGAGCAAGG	ATGATGATGG	900
CTGGATTCAA	TCCAAAACCA	ATGAGTGCTA	AAGTAACCAA	CAATATACAA	AACCTGATAA	960
agcaacaata	TTGCAATAAG	TACAAGCTTA	AAGAAGAAAT	GGGTGAGCTC	CATTTTTGCT	1020
GGGAGGAGAA	AAGCTTAATC	GTTGCTTCAG	CTTGGAGGTA	agataagtga	CAAGAGCATA	1080
TAGTCTTTAT	GTTTCATAAA	ACATAATTAT	GTTTTTACTG	TAATCTTGGG	TTATTGTGTA	1140
ACTGGTTAAA	TCATCTCCAT	GTATTATTAC	CAGAGGTTAG	GGGTGATCAC	AGGTACTARA	1200
AGCTAATCTA	ACACTTATGG	AAGAATTTT	CTTTCTTTTT	TTTCCCTATT	TATAAAAAT	1260
AATTAGAGTT	TTGGTTCTAA	ACCTATTTGC	TAAGTGTGAA	TGAGTCTTTA	CATGTTCATA	1320
TTTCAGTTCA	Antggttaaa	TTTGTTAAGG	TTCTCACTTA	AAAAAAA		1368

(2) INFORMATION FOR SEQ ID NO:23:

- (i) SEQUENCE CHARACTERISTICS:
 - (A) LENGTH: 351 amino acids
 - (B) TYPE: amino acid
 - (C) STRANDEDNESS: unknown (D) TOPOLOGY: unknown

(ii) MOLECULE TYPE: peptide

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:23:

Leu Ser M t Val Asn Glu Leu Arg Gln Ile Val Ser Ile In Gly Asp 5 10 15

Pro Ser Gln Arg Ile Ala Ala Tyr Met Val Glu Gly Leu Ala Ala Arg 20 25 30 Met Ala Ala Ser Gly Lys Phe Ile Tyr Arg Ala Leu Lys Cys Lys Glu 35 40 45 Pro Pro Ser Asp Glu Arg Leu Ala Ala Het Gln Val Leu Phe Glu Val Cys Pro Cys Phe Lys Phe Gly Phe Leu Ala Ala Asn Gly Ala Ile Leu 65 70 75 80 Glu Ala Ile Lys Gly Glu Glu Glu Val His Ile Ile Asp Phe Asp Ile 85 90 95 Asn Gln Gly Asn Gln Tyr Het Thr Leu Ile Arg Ser Ile Ala Glu Leu Pro Gly Lys Arg Pro Arg Leu Arg Leu Thr Gly Ile Asp Asp Pro Glu 115 120 125 Ser Val Gln Arg Ser Ile Gly Gly Leu Arg Ile Ile Asn Leu Arg Leu 130 135 140 Glu Gln Leu Ala Glu Asp Asn Gly Val Ser Phe Lys Phe Lys Ala Met Pro Ser Lys Thr Ser Ile Val Ser Pro Ser Thr Leu Gly Cys Lys Pro Gly Glu Thr Leu Ile Val Asn Phe Ala Phe Gln Leu His His Met Pro Asp Glu Ser Val Thr Thr Val Asn Gln Arg Asp Glu Leu Leu His Het Val Lys Ser Leu Asn Pro Leu Val Thr Val Val Glu Gln Asp Val Asn Thr Asn Thr Ser Pro Phe Phe Pro Arg Phe Ile Glu Ala Tyr Glu Tyr Tyr Ser Ala Val Phe Glu Ser Leu Asp Het Thr Leu Pro Arg Glu Ser Gln Glu Arg Met Asn Val Glu Arg Gln Cys Leu Ala Arg Asp Ile Val 260 265 270 Asn Ile Val Ala Cys Glu Gly Glu Glu Arg Ile Glu Arg Tyr Glu Ala Ala Gly Lys Trp Arg Ala Arg Met Met Met Ala Gly Phe Asn Pro Lys 290 295 300 Pro Met Ser Ala Lys Val Thr Asn Asn Ile Gln Asn Leu Ile Lys Gln Gln Tyr Cys Asn Lys Tyr Lys Leu Lys Glu Glu Met Gly Glu Leu His 325 330 335 Phe Cys Trp Glu Glu Lys Ser Leu Ile Val Ala Ser Ala Trp Arg 345 340

(2) INFORMATION FOR SEQ ID NO: 24:

(i) SEQUENCE CHARACTERISTICS: (A) LENGTH: 100 base pairs

(B)	TYPE: nucleic	acid
(C)	STRANDEDNESS:	single

(D) TOPOLOGY: unknown

(ii) MOLECULE TYPE: cDNA

(xi)	Sequence	DESCRIPTION:	SEQ	ID	NO:24:
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CCAGGAGGCG	TTCGAGCGGG	AGGAGCGTGT	GCACATCATC	GACCTCGACA	TCATGCAGGG	60
GCTGCAGTGG	CCGGGCCTCC	TCCACATCCT	TGCCTCCCGC			100

(2) INFORMATION FOR SEQ ID NO: 25:

- (1) SEQUENCE CHARACTERISTICS:
 - (A) LENGTH: 33 amino acids (B) TYPE: amino acid

 - (C) STRANDEDNESS: unknown (D) TOPOLOGY: unknown

(ii) MOLECULE TYPE: peptide

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:25:

Gln Glu Ala Phe Glu Arg Glu Glu Arg Val His Ile Ile Asp Leu Asp

Ile Met Gln Gly Leu Gln Trp Pro Gly Leu Phe His Ile Leu Ala Ser

Arg

(2) INFORMATION FOR SEQ ID NO:26:

- (i) SEQUENCE CHARACTERISTICS:
 - (A) LENGTH: 1094 base pairs (B) TYPE: nucleic acid

 - (C) STRANDEDNESS: unknown
 - (D) TOPOLOGY: unknown

(ii) MOLECULE TYPE: CDNA

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:26:

CCACGCGTCC	GTCAAAGGAT	ACAACCATGT	ACACATAATT	GACTTTTCCC	TGATGCAAGG	60
TCTCCAGTGG	CCGGCACTCA	TGGATGTCTT	CTCCGCCCGT	GAGGGTGGGC	CACCAAAGCT	120
CCGAATCACA	GGCATTGGCC	CGAACCCAAT	AGGTGGCCGT	GACGAGCTCC	ATGAAGTGGG	180
AATTCGCCTC	GCCAAGTATG	CACACTCGGT	GGGTATCGAC	TTCACTTTCC	AGGGAGTCTG	240
TGTCGATCAA	CTTGATAGGT	TGTGCGACTG	GATGCTTCTC	AAACCAATCA	AAGGAGAGGC	300
AGTTGCCATA						360
AGTGGTGCCC						420

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CTTCACGGTG GTTGAGCATG AGGCAGATCA CAACAGACCA CCACTACTAG AGAGGTTCAC 480 TANTGCCCTC TTCCACTATG CGACCATGTT TGACTCTTTG AGGCCATGC ATCGTTGTAC 540 CAGTGGTAGA GACATCACCG ACTCACTCAC AGAGGTGTAC CTTCGAGGTG AGATTTTTGA 600 CATTGTCTGC GGCGAGGGCA GTGCACGCAC CGAACGTCAT GAGTTGTTTG GTCACTGGAG 660 GGAGAGGCTC ACCTATGCTG GGCTAACTCA AGTGTGGTTC GACCCCGATG AGGTTGACAC 720 GCTAAAAGAC CAGTTGATCC ATGTGACATC CTTATCTGGC TCTGGGTTCA ACATCCTAGT 780 GTGTGATGGC AGCCTTGCAC TAGCGTGGCA TAATCGCCCG TTATATGTGG CAACAGCTTG 840 GTGTGTGACA GGAGGANATG CTGCCAGTTC CATGGTTGGC AACATCTGTA AGGGTACANA 900 TGATAGTAGA AGAAAGGAAA ACCGTAATGG ACCCATGGAG TAGCAGGAAG AATAACCATG 960 TCATGAGCAA ATCGATCAAG TAATAAAATG CACTGATGAC ATGCATGGTG ATCTAAAGTT 1020 TTTTTGCGTG AATGTGCAAT GACGAATTGT TCAATTTGAA TAACCTAATC ATGAGACTCA 1080 1094 AAAA AAAAAAA

(2) INFORMATION FOR SEQ ID NO:27:

- (1) SEQUENCE CHARACTERISTICS:
 - (A) LENGTH: 313 amino acids (B) TYPE: amino acid

 - (C) STRANDEDNESS: unknown
 - (D) TOPOLOGY: unknown
- (ii) MOLECULE TYPE: protein

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:27:

His Ala Ser Val Lys Gly Tyr Asn His Val His Ile Ile Asp Phe Ser

Leu Het Gln Gly Leu Gln Trp Pro Ala Leu Het Asp Val Phe Ser Ala

Arg Glu Gly Gly Pro Pro Lys Leu Arg Ile Thr Gly Ile Gly Pro Asn

Pro Ile Gly Gly Arg Asp Glu Leu His Glu Val Gly Ile Arg Leu Ala

Lys Tyr Ala His Ser Val Gly Ile Asp Phe Thr Phe Gln Gly Val Cys

Val Asp Gln Leu Asp Arg Leu Cys Asp Trp Net Leu Leu Lys Pro Ile

Lys Gly Glu Ala Val Ala Ile Asn Ser Ile Leu Gln Leu His Arg Leu

Leu Val Asp Pro Asp Ala Asn Pro Val Val Pro Ala Pro Ile Asp Ile

L u Leu Lys Leu Val Ile Lys Ile Asn Pro Net Ile Phe Thr Val Val 130 135 140

Glu His Glu Ala Asp His Asn Arg Pro Pr Leu Leu Glu Arg Phe Thr

145					150					155					160
As n	Ala	Leu	Phe	His 165	Tyr	Ala	Thr	Met	Phe 170	yab	Ser	Leu	Glu	Ala 175	Ket
His	Arg	Cys	Thr 180	Ser	Gly	Arg	λsp	11e 185	Thr	Хsр	Ser	Leu	Thr 190	Glu	Val
Tyr	Leu	Arg 195	Gly	Glu	Ile	Phe	Asp 200	Ile	Val	Cys	Gly	Glu 205	Gly	Ser	Ala
Arg	Thr 210	Glu	Arg	His	Glu	Leu 215	Phe	Gly	His	Trp	Arg 220	Glu	Arg	Leu	Thr
Tyr 225	Ala	Gly	Leu	Thr	Gln 230	Val	Trp	Phe	Asp	Pro 235	yeb	Glu	Val	ХБР	Thr 240
Leu	Lys	ysb	Gln	Leu 245	Ile	His	Val	Thr	Ser 250	Leu	Ser	Gly	Ser	Gly 255	Phe
Asn	Ile	Leu	Val 260	Cys	Asp	Gly	Ser	Leu 265	Ala	Leu	Ala	Trp	His 270	Asn	Arg
Pro	Leu	Tyr 275	Val	Ala	Thr	Ala	Trp 280	Cys	Val	Thr	Gly	Gly 285	As n	Ala	Ala
Ser	ser 290	Met	Val	Gly	Asn	Ile 295	Сув	Lys	Gly	Thr	Xan 300	yab	Ser	Arg	Arg
Ly s 305	Glu	As n	Arg	Asn	Gly 310	Pro	Met	Glu							

(2) INFORMATION FOR SEQ ID NO:28:

- (i) SEQUENCE CHARACTERISTICS:
 - (A) LENGTH: 611 base pairs
 (B) TYPE: nucleic acid
 (C) STRANDEDNESS: unknown
 (D) TOPOLOGY: unknown
- (ii) MOLECULE TYPE: DNA (genomic)

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:28:

CCCAACTTGG	GAAGCCCTTC	CTCCGCTCCG	CCTCCTACCT	CAAGGAGGCC	CTCCTCCTCG	60
CACTCGCCGA	CAGCCACCAT	GGCTCCTCCG	GCGTCACCTC	GCCGCTCGAC	GTTGCCCTCA	120
AGCTTGCAGC	ATACAAGTCT	TTCTCTGACC	TGTCACCTGT	GCTCCAGTTC	ACTAACTTTA	180
CCGCAACAAG	GCGCTTCTTG	ATGAGATTGG	TGGCATGGCA	ACTTCCTGCA	TCCATGTCAT	240
TGACTTTGAT	CTCGGTGTTG	GTGGTCAGTG	GGCTTCCTTC	TTGCAGGAGC	TTGCCCACCG	300
CCGGGGAGCT	GGAGGTATGG	CCTTGCCGTT	GTTGAAGCTC	ACGGCTTTCA	TGTCGACTGC	360
TTCTCACCAT	CCACTGGAGC	TGCACCTTAC	CCAGGATAAC	CTCTCTCAGT	TTGCCGCAGA	420
GCTCAGAATT	CCTTTCGAAT	TCAATGCCGT	CAGTCTTGAT	GCATTCAATC	CTGCGGAATC	480
TATTTCTTCC	TCTGGTGATG	AAGTTGTTGC	TGTTAGCCTC	CCTGTTGGCT	GCTCTGCTCG	540
TGCACCACCG	CTGCCAGCGA	TTCTTCGGTT	GGTGAAACAG	CTTTGTCCTA	AGGTTGTCGT	600

PCT/US97/07022

611 GGCTATTGAT C

WO 97/41152

- (1) SEQUENCE CHARACTERISTICS:
 - (A) LENGTH: 502 base pairs

 - (B) TYPE: nucleic acid (C) STRANDEDNESS: unknown
 - (D) TOPOLOGY: unknown

(11) MOLECULE TYPE: CDNA

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:29:

TTTTTTTTT	TTTTTTTTT	TTTTTTTTT	TACAGAGCAA	CAGCAGTATA	ATATTAATTC	60
TGTACCACAC	AACCATTTGA	TAGGTTAAAT	TACCCTCTAG	TCTCTACTCA	TAAGCAGTGT	120
TTCCAATGAG	ATGATCATGG	CTAATTGAGC	AGAGCATGGC	AACAACCTAA	AGCAACATCA	180
TTAGCTATAG	AGACTGACAC	CAATATTCCT	AAATCCACTA	GGCTAGCTAA	TAAGCTGCAA	240
CGAAAAGCAA	TATGAAGAGT	TCAACAGCTC	AAGACAACAA	TTTCATTTGC	AACATTTAAT	300
T CAAGAATA	AATGGACATT	ACTGGAGTGG	TCGATGCTTG	CAAACGGTGG	TGGAACCTTG	360
GTGGAGTGAA	GCTTATGGCT	GATCAGCACC	GCCAAGATGA	TATGGATACA	AGCTCCCCAC	420
GCTGCCAGTA	GAGCGTAAGA	GCAGCTCCGC	GTTTCTCCAC	ATGGAATCCT	CGGACCTGCA	480
CCCGCTTCAG	GAGGCAGTCT	GC				502

(2) INFORMATION FOR SEQ ID NO:30:

- (i) SEQUENCE CHARACTERISTICS:
 - (A) LENGTH: 298 amino acids
 - (B) TYPE: amino acid
 - (C) STRANDEDNESS: single
 - (D) TOPOLOGY: unknown
- (ii) MOLECULE TYPE: peptide

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:30:

Pro Gln Gln Gln Gln His Gln Gln Gln Gln Gln His Lys Pro

Pro Pro Pro Pro Ile Gln Gln Gln Glu Arg Glu Asn Ser Ser Thr Asp

Ala Pro Pro Gln Pro Glu Thr Val Thr Ala Thr Val Pro Ala Val Gln

Thr Asn Thr Ala Glu Ala Leu Arg Glu Arg Lys Glu Glu Ile Lys Arg

Gln Lys Gln Asp lu Glu Gly Leu His Leu Leu Thr Leu Leu Cln

Cys Ala Glu Ala Val Ser Ala Asp Asn Leu Glu Glu Ala Asn Lys Leu

Leu Leu Glu Ile Ser Gln Leu Ser Thr Pro Tyr ly Thr Ser Ala ln 100 105

Arg Val Ala Ala Tyr Phe Ser Glu Ala Het Ser Ala Arg Leu Leu Asn

ser Cys Leu Gly Ile Tyr Ala Ala Leu Pro Ser Arg Trp Het Pro Gln

Thr His Ser Leu Lys Met Val Ser Ala Phe Gln Val Phe Asn Gly Ile 150

Ser Pro Leu Val Lys Phe Ser His Phe Thr Ala Asn Gln Ala Ile Gln

Glu Ala Phe Glu Lys Glu Asp Ser Val His Ile Ile Asp Leu Asp Ile

Met Gln Gly Leu Gln Trp Pro Gly Leu Phe His Ile Leu Ala Ser Arg 200

Pro Gly Gly Pro Pro His Val Arg Leu Thr Gly Leu Gly Thr Ser Met 210 220

Glu Ala Leu Gln Ala Thr Gly Lys Arg Leu Ser Asp Phe Thr Asp Lys

Leu Gly Leu Pro Phe Glu Phe Cys Pro Leu Ala Glu Lys Val Gly Asn 250

Asp Leu Thr Glu Arg Leu Asn Val Arg Lys Arg Glu Ala Ala Val His

Trp Leu Gln His Ser Leu Tyr Asp Val Thr Gly Ser Asp Ala His Thr

Leu Trp Leu Leu Gln Arg Leu Ala Pro Lys

(2) INFORMATION FOR SEQ ID NO:31:

- (i) SEQUENCE CHARACTERISTICS:
 - (A) LENGTH: 307 amino acids
 - (B) TYPE: amino acid
 - (C) STRANDEDNESS: single (D) TOPOLOGY: unknown

(ii) MOLECULE TYPE: peptide

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:31:

Gly Thr Ser Pro Thr Gly Pro Glu Leu Leu Thr Tyr Met His Ile Leu

Tyr Glu Ala Cys Pro Tyr Phe Lys Phe Gly Tyr Glu Ser Ala Asn Gly 20 25 30

Ala Ile Ala Glu Ala Val Lys Asn Glu Ser Phe Val His Ile Ile Asp

Phe Gin Ile Ser Gin Gly Gly in Trp Val Ser Leu Ile Arg Ala Leu

Gly Ala Arg Pr Gly Gly Pro Pro Asn Val Arg Ile Thr Gly Ile Asp

70 75 80 Asp Pro Arg Ser Ser Phe Ala Arg In Gly Gly Leu Glu Leu Val Gly Gln Arg Leu Gly Lys Leu Ala Glu Het Cys Gly Val Pro Phe Glu Phe His Gly Ala Ala Leu Cys Cys Thr Glu Val Glu Ile Glu Lys Leu Gly Val Arg Asn Gly Glu Ala Leu Ala Val Asn Phe Pro Leu Val Leu His His Met Pro Asp Glu Ser Val Thr Val Glu Asn His Arg Asp Arg Leu Leu Arg Leu Val Lys His Leu Ser Pro Asn Val Val Thr Leu Val Glu 165 170 175 Gln Glu Ala Asn Thr Asn Thr Ala Pro Phe Leu Pro Arg Phe Val Glu Thr Met Asn His Tyr Leu Ala Val Phe Glu Ser Ile Asp Val Lys Leu Ala Arg Asp His Lys Glu Arg Ile Asn Val Glu Gln His Cys Leu Ala Arg Glu Val Val Asn Leu Ile Ala Cys Glu Gly Val Glu Arg Glu Glu 225 230 235 240 Arg His Glu Pro Leu Gly Lys Trp Arg Ser Arg Phe His Met Ala Gly Phe Lys Pro Tyr Pro Leu Ser Ser Tyr Val Asn Ala Thr Ile Lys Gly 260 265 270 Leu Leu Glu Ser Tyr Ser Glu Lys Tyr Thr Leu Glu Glu Arg Asp Gly Ala Leu Tyr Leu Gly Trp Lys Asn Gln Pro Leu Ile Thr Ser Cys Ala Trp Arg Xaa 305

(2) INFORMATION FOR SEQ ID NO: 32:

- (i) SEQUENCE CHARACTERISTICS:
 - (A) LENGTH: 353 amino acids
 - (B) TYPE: amino acid
 - (C) STRANDEDNESS: single
 - (D) TOPOLOGY: unknown
- (ii) MOLECULE TYPE: peptide

(x1) SEQUENCE DESCRIPTION: SEQ ID NO:32:

Leu Ser Met Val Asn Glu Leu Arg Gln Ile Val Ser Ile Gln Gly Asp

Pro Ser Gln Arg Ile Ala Ala Tyr Met Val Glu Gly Leu Ala Ala Arg 20 25 30

Met Ala Ala Ser Gly Lys Phe Ile Tyr Arg Ala Leu Lys Cys Lys Glu 35 40 45 Pro Pr Ser Asp Glu Arg Leu Ala Ala Met Gln Val Leu Phe Glu Val Cys Pro Cys Phe Lys Phe Gly Phe Leu Ala Ala Asn Gly Ala Ile Leu 65 70 75 80 Glu Ala Ile Lys Gly Glu Glu Glu Val His Ile Ile Asp Phe Asp Ile Asn Gin Gly Asn Gin Tyr Met Thr Leu Ile Arg Ser Ile Ala Glu Leu 100 105 110 Pro Gly Lys Arg Pro Arg Leu Arg Leu Thr Gly Ile Asp Asp Pro Glu 115 120 125 Ser Val Gln Arg Ser Ile Gly Gly Leu Arg Ile Ile Gly Leu Arg Leu 130 135 140 Glu Gln Leu Ala Glu Asp Asn Gly Val Ser Phe Lys Phe Lys Ala Met Pro Ser Lys Thr Ser Ile Val Ser Pro Ser Thr Leu Gly Cys Lys Pro Gly Glu Thr Leu Ile Val Asn Phe Ala Phe Gln Leu His His Met Pro Amp Glu Ser Val Thr Thr Val Am Gln Arg Amp Glu Leu Leu Him Met 200 Val Lys Ser Leu Asn Pro Lys Leu Val Thr Val Val Glu Gln Asp Val Asn Thr Asn Thr Ser Pro Phe Phe Pro Arg Phe Ile Glu Ala Tyr Glu Tyr Tyr Ser Ala Val Phe Glu Ser Leu Asp Met Thr Leu Pro Arg Glu Ser Gln Glu Arg Met Asn Val Glu Arg Gln Cys Leu Ala Arg Asp Ile Val Asn Ile Val Ala Cys Glu Gly Glu Glu Arg Ile Glu Arg Tyr Glu 275 280 285 Ala Ala Gly Lys Trp Arg Ala Arg Met Het Het Ala Gly Phe Asn Pro 295 Lys Pro Met Ser Ala Lys Val Thr Asn Asn Ile Gln Asn Leu Ile Lys Gin Gin Tyr Cys Asn Lys Tyr Lys Leu Lys Glu Glu Met Gly Glu Leu His Phe Cys Trp Glu Glu Lys Ser Leu Ile Val Ala Ser Ala Trp Arg 345

- Xaa
- (2) INFORMATION FOR SEQ ID NO:33:
 - (i) SEQUENCE CHARACTERISTICS: (A) LENGTH: 326 amino acids

- (B) TYPE: amino acid
- (C) STRANDEDNESS: single
- (D) TOPOLOGY: unknown

(ii) MOLECULE TYPE: peptide

(x1) SEQUENCE DESCRIPTION: SEQ ID NO:33:

Ala Het Glu Gly Glu Lys Het Val His Val Ile Asp Leu Asp Ala Ser Glu Pro Ala Gln Trp Leu Ala Leu Leu Gln Ala Phe Asn Ser Arg Pro 20 25 30 Glu Gly Pro Pro His Leu Arg Ile Thr Gly Val His His Gln Lys Glu 35 40 45 Val Leu Glu Gln Met Ala His Arg Leu Ile Glu Glu Ala Glu Lys Leu Amp Ile Pro Phe Gln Phe Amn Pro Val Val Ser Arg Leu Amp Cym Leu Asn Val Glu Gln Leu Arg Val Lys Thr Gly Glu Ala Leu Ala Val Ser Ser Val Leu Gln Leu His Thr Phe Leu Ala Ser Asp Asp Leu Met Arg Lys Asn Cys Ala Leu Arg Phe Gln Asn Asn Pro Ser Gly Val Asp Leu Gln Arg Val Leu Het Het Ser His Gly Ser Ala Ala Glu Ala Arg Glu Asn Asp Het Ser Asn Asn Gly Tyr Ser Pro Ser Gly Asp Ser Ala Ser Ser Leu Pro Leu Pro Ser Ser Gly Arg Thr Asp Ser Phe Leu 165 170 175 Asn Ala Ile Trp Gly Leu Ser Pro Lys Val Het Val Val Thr Glu Gln Asp Ser Asp His Asn Gly Ser Thr Leu Met Glu Arg Leu Glu Ser 195 200 205 Leu Tyr Thr Tyr Ala Ala Leu Phe Asp Cys Leu Glu Thr Lys Val Pro Arg Thr Ser Gln Asp Arg Ile Lys Val Glu Lys Met Leu Phe Gly Glu 225 230 235 240 Glu Ile Lys Asn Ile Ile Ser Cys Glu Gly Phe Glu Arg Arg Glu Arg 245 250 255 His Glu Lys Leu Glu Lys Trp Ser Gln Arg Ile Asp Leu Ala Gly Phe 265 Gly Asn Val Pro Leu Ser Tyr Tyr Ala Met Leu Gln Ala Arg Arg Leu

Leu Gin Gly Cys Gly Phe Asp Gly Tyr Arg Ile Lys Glu Glu Ser Gly

295

Cys Ala Val Ile Cys Trp Gln Asp Arg Pr Leu Tyr Ser Val Ser Ala

Trp Arg Cys Arg Lys Xaa

(2) INFORMATION FOR SEQ ID NO: 34:

- (1) SEQUENCE CHARACTERISTICS:
 - (A) LENGTH: 277 amino acids
 - (B) TYPE: amino acid
 - (C) STRANDEDNESS: single (D) TOPOLOGY: unknown

(ii) MOLECULE TYPE: peptide

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:34:

Asn Lys Arg Lau Lys Ser Cys Ser Ser Pro Asp Ser Met Val Thr Ser

Thr Ser Thr Gly Thr Gln Ile Gly Gly Val Ile Gly Thr Thr Val Thr 20 25 30

Thr Thr Thr Thr Thr Thr Ala Ala Ala Glu Ser Thr Arg Ser Val

Ile Leu Val Asp Ser Gln Glu Asn Gly Val Arg Leu Val His Ala Leu

Het Ala Cys Ala Glu Ala Ile Gln Gln Asn Asn Leu Thr Leu Ala Glu 65 70 75 80

Ala Leu Val Lys Gln Ile Gly Cys Leu Ala Val Ser Gln Ala Gly Ala 85 90 95

Het Arg Lys Val Ala Thr Tyr Phe Ala Glu Ala Leu Ala Arg Arg Ile

Tyr Arg Leu Ser Pro Pro Gln Asn Gln Ile Asp His Cys Leu Ser Asp

Thr Leu Gln Met His Phe Tyr Glu Thr Cys Pro Tyr Leu Lys Phe Ala 130 135 140

His Phe Thr Ala Asn Gln Ala Ile Leu Glu Ala Phe Glu Gly Lys Lys

Arg Val His Val Ile Asp Phe Ser Het Asn Gln Gly Leu Gln Trp Pro

Ala Leu Met Gln Ala Leu Ala Leu Arg Glu Gly Gly Pro Pro Thr Phe

Arg Leu Thr Gly Ile Gly Pro Pro Ala Pro Asp Asn Ser Asp His Leu 200

His Glu Val Gly Cys Lys Leu Ala Gln Leu Ala Glu Ala Ile His Val

lu Tyr Arg Gly Phe Val Ala Asn Ser Leu Ala Asp Leu Asp

Ala Ser Met Leu Glu Leu Arg Pr S r Asp Thr Glu Ala Val Ala Val

255

250

Asn Ser Val Phe Glu Leu His Lys Leu Leu Gly Arg Xaa Gly Gly Ile 260 265 270

Glu Lys Val Leu Gly 275

(2) INFORMATION FOR SEQ ID NO:35:

- (i) SEQUENCE CHARACTERISTICS:
 - (A) LENGTH: 262 amino acids

245

- (B) TYPE: amino acid
- (C) STRANDEDNESS: single
- (D) TOPOLOGY: unknown
- (ii) MOLECULE TYPE: peptide

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:35:

Gly Gly Gly Asp Thr Tyr Thr Thr Asn Lys Arg Leu Lys Cys Ser 1 10 15

Asn Gly Val Val Glu Thr Thr Thr Ala Thr Ala Glu Ser Thr Arg His 20 25 30

Val Val Leu Val Asp Ser Gln Glu Asn Gly Val Arg Leu Val His Ala 35 40 45

Leu Leu Ala Cys Ala Glu Ala Val Gln Lys Glu Asn Leu Thr Val Ala 50 55 60

Glu Ala Leu Val Lys Gln Ile Gly Phe Leu Ala Val Ser Gln Ile Gly 65 70 75 80

Ala Met Arg Gln Val Ala Thr Tyr Phe Ala Glu Ala Leu Ala Arg Arg 85 90 95

Ile Tyr Arg Leu Ser Pro Ser Gln Ser Pro Ile Asp His Ser Leu Ser 100 105 110

Asp Thr Leu Gln Met His Phe Tyr Glu Thr Cys Pro Tyr Leu Lys Phe 115 120 125

Ala His Phe Thr Ala Asn Gln Ala Ile Leu Glu Ala Phe Gln Gly Lys 130 135 140

Lys Arg Val His Val Ile Asp Pha Ser Met Ser Gln Gly Leu Gln Trp 145 150 155 160

Pro Ala Leu Met Gln Ala Leu Ala Leu Arg Pro Gly Gly Pro Pro Val 165 170 175

Phe Arg Leu Thr Gly Ile Gly Pro Pro Ala Pro Asp Asn Phe Asp Tyr 180 185 190

Leu His Glu Val Gly Cys Lys Leu Ala His Leu Ala Glu Ala Ile His 195 200 205

Val Glu Phe Glu Tyr Arg Gly Phe Val Ala Asn Thr Leu Ala Asp Leu 210 215 220

Asp Ala Ser Met Leu Glu Leu Arg Pro Ser Glu Ile Glu Ser Val Ala 225 230 235 240

Val Asn Ser Val Phe Glu Leu His Lys Leu Leu Gly Arg Pr Gly Ala 245 250 255

Ile Asp Lys Val Leu Gly 260

(2) INFORMATION FOR SEQ ID NO:36:

- (1) SEQUENCE CHARACTERISTICS:
 - (A) LENGTH: 203 amino acids
 - (B) TYPE: amino acid
 - (C) STRANDEDNESS: single
 - (D) TOPOLOGY: unknown

(ii) MOLECULE TYPE: peptide

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:36:

Gin Leu Gly Lys Pro Phe Leu Arg Ser Ala Ser Tyr Leu Lys Glu Ala 1 10 15

Leu Leu Ala Leu Ala Asp Ser His His Gly Ser Ser Gly Val Thr
20 25 30

Ser Pro Leu Asp Val Ala Leu Lys Leu Ala Ala Tyr Lys Ser Phe Ser 35 40 45

Asp Leu Ser Pro Val Leu Gln Phe Thr Asn Phe Thr Ala Asn Lys Ala 50 55 60

Leu Leu Asp Glu Ile Gly Gly Met Ala Thr Ser Cys Ile His Val Ile 65 70 75 80

Asp Phe Asn Leu Gly Val Gly Gly Gln Trp Ala Ser Phe Leu Gln Glu 85 90 95

Leu Ala His Arg Arg Gly Ala Gly Gly Met Ala Leu Pro Leu Leu Lys 100 105 110

Leu Thr Ala Phe Het Ser Thr Ala Ser His His Pro Leu Glu Leu His 115 120 125

Leu Thr Gln Asp Asn Leu Ser Gln Phe Ala Ala Glu Leu Arg Ile Pro 130 135 140

Phe Glu Phe Asn Ala Val Ser Leu Asp Ala Phe Asn Pro Ala Glu Ser 145 150 155 160

Ile Ser Ser Ser Gly Amp Glu Val Val Ala Val Ser Leu Pro Val Gly
165 170 175

Cys Ser Ala Arg Ala Pro Pro Leu Pro Ala Ile Leu Arg Leu Val Lys 180 185 190

Gin Leu Cys Pro Lys Val Val Val Ala Ile Asp 195 200

(2) INFORMATION FOR SEQ ID NO: 37:

(1) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 131 amin acids
- (B) TYPE: amin acid
- (C) STRANDEDNESS: single
- (D) TOPOLOGY: unknown

(11) MOLECULE TYPE: peptide

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:37:

His Ala Ser Val Lys Gly Tyr Asn His Val His Ile Ile Asp Phe Ser

Leu Met Gln Gly Leu Gln Trp Pro Ala Leu Het Asp Val Phe Ser Ala

Arg Glu Gly Gly Pro Pro Lys Leu Arg Ile Thr Gly Ile Gly Pro Asn

Pro Ile Gly Gly Arg Asp Glu Leu His Glu Val Gly Ile Arg Leu Ala

Lys Tyr Ala His Ser Val Gly Ile Asp Phe Thr Phe Gln Gly Val Cys

Val Asp Gln Leu Asp Arg Leu Cys Asp Trp Het Leu Leu Lys Pro Ile

Lys Gly Glu Ala Val Ala Ile Asn Ser Ile Leu Gln Leu His Arg Leu

Leu Val Asp Pro Asp Ala Asn Pro Val Val Pro Ala Pro Ile Asp Ile 115

Leu Leu Lys 130

(2) INFORMATION FOR SEQ ID NO:38:

- (i) SEQUENCE CHARACTERISTICS:
 - (A) LENGTH: 33 amino acids (B) TYPE: amino acid

 - (C) STRANDEDNESS: single
 - (D) TOPOLOGY: unknown
- (ii) MOLECULE TYPE: peptide
- (xi) SEQUENCE DESCRIPTION: SEQ ID NO:38:

Gln Glu Ala Phe Glu Arg Glu Glu Arg Val His Ile Ile Asp Leu Asp

Ile Met Gin Gly Leu Gin Trp Pro Gly Leu Phe His Ile Leu Ala Ser

Arg

(2) INFORMATION FOR SEQ ID NO:39:

- (1) SEQUENCE CHARACTERISTICS:
 - (A) LENGTH: 29 amino acids
 - (B) TYPE: amin acid
 - (C) STRANDEDNESS: singl
 - (D) TOPOLOGY: unknown
- (ii) MOLECULE TYPE: peptide

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:39:

Phe Ala Gly Cys Arg Arg Val His Val Val Asp Phe Gly Ile Lys Gln
1 10 15

Gly Met Gln Trp Pro Ala Leu Leu Xaa Asp Leu Ala Leu 20 25

- (2) INFORMATION FOR SEQ ID NO: 40:
 - (i) SEQUENCE CHARACTERISTICS:
 - (A) LENGTH: 73 amino acids
 - (B) TYPE: amino acid
 - (C) STRANDEDNESS: single.
 - (D) TOPOLOGY: unknown
 - (ii) MOLECULE TYPE: peptide
 - (xi) SEQUENCE DESCRIPTION: SEQ ID NO:40:
 - Gly Arg Asn Gly Arg Thr Leu Trp Leu Gly Glu Gly His Ile Asp Leu
 1 10 15
 - Trp Pro Leu Gln Gly Leu Leu Ser Gln Gly Leu Gln Arg Ala Leu Cys 20 25 30
 - Ala Arg Pro Leu Gly Ala Pro His Val Phe Leu Pro Gly Leu His Thr 35 40 45
 - Leu Ser Leu Gly Leu Gln Xaa Arg His Leu Leu Val His Het Met Ala 50 55 60
 - Leu Ser Tyr Ser Tyr Gly Arg Xaa Pro
- (2) INFORMATION FOR SEQ ID NO:41:
 - (i) SEQUENCE CHARACTERISTICS:
 - (A) LENGTH: 59 amino acids
 - (B) TYPE: amino acid
 - (C) STRANDEDNESS: single
 - (D) TOPOLOGY: unknown
 - (ii) MOLECULE TYPE: peptide
 - (x1) SEQUENCE DESCRIPTION: SEQ ID NO:41:
 - Thr Ser Asp Ser Ala Ser Ser Phe Asn Ile Pro Thr Ser Ala Gln Asn 1 5 10 15
 - His Tyr Ala Thr Gly Ser Phe Ser Thr Asn Ser Arg Thr Thr Asn Val 20 25 30
 - Ala Thr Ala Thr Thr Asn Ser Ala Thr Ala His Trp Val Ala Thr Asp
 35 40 45
 - Ala Glu His Thr Asp Thr Ile Il Ala Gln Pr 50 55

(2) INFORMATION FOR SEQ ID NO: 42:

- (1) SEQUENCE CHARACTERISTICS:
 - (A) LENGTH: 110 amin acids
 - (B) TYPE: amin acid
 - (C) STRANDEDNESS: single (D) TOPOLOGY: unknown
- (ii) MOLECULE TYPE: peptide

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 42:

Arg Xaa Phe Asp Ser Leu Glu His Asp Ala Ser Lys Gly Glu Pro Arg

Glu Asp Glu Arg Gly Arg Xaa Cys Leu Ala Arg Asn Ile Val Asn Ile 20 25 30

Val Xaa Cys Lys Xaa Glu Glu Arg Ile Glu Arg Tyr Glu Val Thr Gly

Lys Trp Arg Ala Arg Met Met Met Ala Gly Phe Ser Pro Arg Pro Met

Ser Gly Arg Val Thr Ser Asn Ile Glu Ser Leu Ile Lys Arg Asp Tyr
65 70 75 80

Cys Ser Lys Tyr Lys Val Lys Glu Glu Met Gly Glu Leu His Phe Ser

Trp Glu Glu Lys Ser Leu Ile Val Ala Ser Ala Trp Ser Xaa 100

(2) INFORMATION FOR SEQ ID NO:43:

- (i) SEQUENCE CHARACTERISTICS:
 - (A) LENGTH: 137 amino acids

 - (B) TYPE: amino acid (C) STRANDEDNESS: single
 - (D) TOPOLOGY: unknown
- (ii) MOLECULE TYPE: peptide

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:43:

Asn Gly Ser Tyr Asn Ala Pro Phe Phe Val Thr Arg Phe Arg Glu Ala

Leu Phe His Tyr Ser Ala Ile Phe Asp Net Leu Glu Thr Asn Ile Pro

Lys Asp Asn Glu Gln Arg Leu Leu Ile Glu Ser Ala Leu Phe Ser Arg

Glu Xaa Asn Val Ile Ser Cys Glu Gly Leu Glu Arg Het Glu Arg Pro

Glu Thr Tyr Lys Gln Trp Gln Val Arg Asn Gln Arg Val Gly Phe Lys 65 70 75 80

Gin Leu Pro L u Asn Gin Asp Met Het Lys Arg Ala Arg Xaa Glu ly

> 85 90 95

Gln Val Leu Pro Thr Arg Thr Phe Ile Ile Asp Glu Asp Asn Arg Trp 100 105

Lau Lau Gln Gly Trp Lys Gly Arg Ile Lau Phe Ala Lau Ser Thr Trp 125

Lys Pro Asp Asn Arg Ser Ser Ser Xaa 130

(2) INFORMATION FOR SEQ ID NO:44:

- (i) SEQUENCE CHARACTERISTICS:
 - (A) LENGTH: 41 amino acids
 - (B) TYPE: amino acid
 - (C) STRANDEDNESS: single (D) TOPOLOGY: unknown
- (ii) MOLECULE TYPE: peptide

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:44:

Asn Gly Gly Ala Phe Ala Pro Ser Thr Trp Thr Ala Arg Ser Leu Asn

Gly Gly Ala Phe Ala Pro Ser Thr Trp Thr Ala Arg Ser Leu Pro Val 25

Pro Ser Ser Pro Ser Thr Asp Ser Phe

(2) INFORMATION FOR SEQ ID NO:45:

- (i) SEQUENCE CHARACTERISTICS:
 - (A) LENGTH: 1279 base pairs
 - (B) TYPE: nucleic acid
 - (C) STRANDEDNESS: unknown
 - (D) TOPOLOGY: unknown

(ii) MOLECULE TYPE: CDNA

(x1) SEQUENCE DESCRIPTION: SEQ ID NO:45:

GCGGCTATCT TCTACGGCCA CCACCACCAT ACACCTCCGC CGGCAAAGCG GCTCAACCCT 60 GGTCCCGTGG GGATAACAGA GCAGCTGGTT AAGGCAGCAG AGGTCATAGA GAGCGACACG 120 TGTCTAGCTC AGGGGATATT GGCGCGGCTC AATCAACAGC TCTCTTCTCC CGTCGGGAAG 180 CCATTAGAAA GAGCAGCTTT TTACTTCAAA GAAGCTCTCA ATAATCTCCT TCACAACGTC 240 TCCCAAACCC TAAACCCTTA TTCCCTCATC TTCAAGATCG CTGCTTACAA ATCCTTCTCA 300 GAGATETETE CEGITETTEA GITEGECAAC TITACETECA ACCAAGECET CITAGAGICE 360 TTCCATGGCT TCCACCGTCT CCACATCATC GACTTCGATA TCGGCTACG TGGCCAATGG 420 GCTTCCCTCA TGCAAGAGCT TGTTCTCCGC GACAACGCCG CTCCTCTCTC CCTCAAGATC 480 ACCOTTTCG CTTCTCCGGC AACCACGAC CAGCTCGAAC TTGGCTTCAC TCAAGACAAC 540

CTCAA CACT TOGCCTCTGA GATCAACATC TCCCTTGACA TCCAAGTTTT GAGCTTAGAC 600 CTCCTCGGCT CCATCTCGTG GCCTAACTCG TCGGAGAAAG AAGCTGTCGC CGTTAACATC 660 TCCGCCGCGT CCTTCTCGCA CCTCCCTTTG GTCCTCCGTT TCGTGAAGCA TCTATCTCCG 720 ACGATCATCG TCTGCTCCGA CAGAGGATGC GAGAGGACGG ATCTGCCCTT CTCTCAACAG 780 CTCGCCCACT CGCTGCACTC ACACACCGCT CTCTTCGAAT CCCTCGACGC CGTCAACGCC 840 AACCTCGACG CAATGCAGAA GATCGAGAGG TTTCTTATAC AGCCGGAGAT AGAGAAGCTG 900 GTGTTGGATC GTAGCCGTCC GATAGAAAGG CCGATGATGA CGTGGCAAGC GATGTTTCTA 960 CAGATGGGTT TCTCACCGGT GACGCACAGT AACTTCACGG AGTCTCAAGC CGAGTGTTTA 1020 GTCCANCGGA CGCCAGTGAG AGGCTTTCAC GTCGAGAAGA AACATAACTC ACTTCTCCTA 1080 TGTTGGCAAA GGACAGAACT CGTCGGAGTT TCAGCATGGA GATGTCGCTC CTCCTGATTT 1140 CCACCGGAGT TTCAATTATT AAAAAATAT TTTCCTTAAT TCAATTTATC TTAAATGACA 1200 ARTITITAGI TICIGATITI ATTITGCTCA GIGCGAIGGA TITTIAARIT TARGITICAC 1260 1279 ACAAATATAT AAATTTTTG

(2) INFORMATION FOR SEQ ID NO: 46:

- (i) SEQUENCE CHARACTERISTICS:
 - (A) LENGTH: 379 amino acide
 - (B) TYPE: amino acid
 - (C) STRANDEDNESS: single
 - (D) TOPOLOGY: unknown

(ii) MOLECULE TYPE: peptide

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 46:

Ala Ala Ile Phe Tyr Gly His His His His Thr Pro Pro Pro Ala Lys

Arg Leu Asn Pro Gly Pro Val Gly Ile Thr Glu Gln Leu Val Lys Ala 20 25 30

Ala Glu Val Ile Glu Ser Asp Thr Cys Leu Ala Gln Gly Ile Leu Ala 35 40 45

Arg Leu Asn Gln Gln Leu Ser Ser Pro Val Gly Lys Pro Leu Glu Arg

Ala Ala Phe Tyr Phe Lys Glu Ala Leu Asn Asn Leu Leu His Asn Val 65 70 75

Ser Gln Thr Leu Asn Pro Tyr Ser Leu Ile Phe Lys Ile Ala Ala Tyr 85 90 95

Lye Ser Phe Ser Glu Ile Ser Pro Val Leu Gln Phe Ala Asn Phe Thr 100 105 110

Ser Asn Gln Ala Leu Leu lu Ser Phe His Gly Phe His Arg Leu His 115 120 125

Ile Ile Asp Phe Asp Ile Gly Tyr Gly Gly In Trp Ala Ser Leu Met 130 135 140

Gln 145	lu	Leu	Val	Leu	Arg 150	Хsр	λsn	Ala	Ala	Pr 155		Ser	Leu	Lys	I1 160
Thr	Val	Phe	Ala	8er 165	Pr	Ala	yeu	His	As p 170	Gln	Leu	Glu	Leu	G1ÿ 175	Phe
Thr	Gln	Asp	Asn 180	Leu	Lye	His	Phe	Ala 185	Ser	Glu	lle	As n	Ile 190	Ser	Leu
Asp	Ile	Gln 195	Val	Leu	Ser	Leu	Asp 200	Leu	Leu	Gly	Ser	Ile 205	Ser	Trp	Pro
Asn	Ser 210	Ser	Glu	Lys	Glu	Ala 215	Val	Ala	Val	Asn	Ile 220	Ser	Ala	Ala	Ser
Phe 225	Ser	His	Leu	Pro	Leu 230	Val	Leu	Arg	Phe	Val 235	Lys	His	Leu	Ser	Pro 240
Thr	Ile	Ile	Val	Cys 245	Ser	Х в р	Arg	Gly	Cys 250	Glu	Arg	Thr	Asp	Leu 255	Pro
Phe	Ser	Gln	Gln 260	Leu	Ala	His	Ser	Leu 265	Hip	Ser	His	Thr	Ala 270	Leu	Phe
Glu	Ser	Leu 275	Хsр	Ala	Val	Xe n	Ala 280	As n	Leu	Asp	Ala	Met 285	Gln	Lys	Ile
Glu	Arg 290	Phe	Leu	Ile	Gln	Pro 295	Glu	Ile	Glu	Lys	Leu 300	Val	Leu	Asp	Arg
Ser 305	Arg	Pro	Ile	Glu	Arg 310	Pro	Met	Met	Thr	Trp 315	Gln	Ala	Xet	Phe	Leu 320
Gln	Met	Gly	Phe	Ser 32 5	Pro	Val	Thr	His	Ser 330	N an	Phe	Thr	Glu	Ser 335	Gln
Ala	Glu	Cys	Leu 340	Val	Gln	Arg	Thr	Pro 345	Val	Arg	Gly	Phe	His 350	Val	Glu
Lys	Lys	His 355	λsn	Ser	Leu	Leu	Leu 360	Cys	Trp	Gln	Arg	Thr 365	Glu	Leu	Va1
Gly	Val 370	Ser	Ala	Trp	Arg	Cys 375	Arg	Ser	Ser	Xaa					

(2) INFORMATION FOR SEQ ID NO:47:

(i) SEQUENCE CHARACTERISTICS:

(A) LENGTH: 745 base pairs

(B) TYPE: nucleic acid

(C) STRANDEDNESS: unknown

(D) TOPOLOGY: unknown

(ii) MOLECULE TYPE: cDNA

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:47:

TGCATACAAC	GCACCGTTTT	TCGTAACACG	GTTTCGCGAA	GCTCTATTTC	ATTTCTCCTC	60
Gatttttgac	ATGCTTGAGA	CAATTGTGCC	ACGAGAAGAC	GAAGAGAGGA	TGTTCCTTGA	120
GATGGAGGTC	TTTGGGAGAG	AGGCACTGAA	TGTGATTGCT	TGCGAAGGTT	GGGAAAGAGT	180
GGAGAGGCCT	GAGACATACA	AGCAGTGGCA	CGTACGGGCT	ATGAGGTCAG	GGTTGGTGCA	240

GGTTCCATT	IT GACCCAAGCA	TTATGAAGAC	ATCCCTCCAT	AAGGTCCACA	CATTCTACCA	300
CAAGGATT	TT GTGATCGATC	AAGATAACCG	GTGGCTCTTG	CAAGGCTG A	AGGGAAGAAC	360
TGTCATGG	CT CTTTCTGTTT	GGAAACCAGA	GTCCAAGGCT	TGACCGAGAA	ATCCTCGTTG	420
GCATATGA	ga gaccatetet	TGATTTTCTT	CCTGTGTAAT	TCCCAGAGAC	AGAATTACAG	480
ATGTAAGAI	ng agaatgetge	ACAAAGAACT	TGTTCAAAGA	TAATATTGAT	GTAAGTCCTG	540
TTTTATAA	CT TTCTAGCTGT	GTTTTTGTTG	TTTCTCAGCT	AGATTCTCCT	AACGGTATTC	600
TTGTAGCT	ng ggtgatcaga	TIGITIGIAT	ATTGCTAGCA	GAGTTAGTTT	GTCTAGATTG	660
TANCACAT	AT AAGAGGAAGC	TTAGAGTTTC	TATGGTTTAA	AGAGAAGTTT	TTTCCTTCTC	720
CARTGTAN	** *******	****				745

(2) INFORMATION FOR SEQ ID NO:48:

- (i) SEQUENCE CHARACTERISTICS:
 - (A) LENGTH: 134 amino acids
 - (B) TYPE: amino acid
 - (C) STRANDEDNESS: single
 - (D) TOPOLOGY: unknown

(ii) MOLECULE TYPE: peptide

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:48:

Ala Tyr Asn Ala Pro Phe Phe Val Thr Arg Phe Arg Glu Ala Leu Phe

His Phe Ser Ser Ile Phe Asp Met Leu Glu Thr Ile Val Pro Arg Glu

Asp Glu Glu Arg Met Phe Leu Glu Met Glu Val Phe Gly Arg Glu Ala

Leu Asn Val Ile Ala Cys Glu Gly Trp Glu Arg Val Glu Arg Pro Glu

Thr Tyr Lys Gln Trp His Val Arg Ala Het Arg Ser Gly Leu Val Gln

Val Pro Phe Asp Pro Ser Ile Het Lys Thr Ser Leu His Lys Val His

Thr Phe Tyr His Lys Asp Phe Val Ile Asp Gln Asp Asn Arg Trp Leu

Leu Gln Gly Trp Lys Gly Arg Thr Val Het Ala Leu Ser Val Trp Lys 120

Pro Glu Ser Lys Ala Xaa 130

(2) INFORMATION FOR SEQ ID NO: 49:

- (1) SEQUENCE CHARACTERISTICS:
 - (A) LENGTH: 775 base pairs
 (B) TYPE: nucl ic acid
 (C) STRANDEDNESS: unknown
 (D) TOPOLOGY: unknown

(11) MOLECULE TYPE: CDNA

(xi)	SEOUE	NCE	DESCRIPTION:	SEO	TO N	.49

λN	LAAATG GG	AAACCATCAC	TCTTGATGAA	CTTATGATCA	ATCCAGGAGA	GACAACGGTC	60
GT	CAACTGCA	TTCATCGGTT	ACAATACACT	CCTGATGAAA	CTGTGTCATT	AGACTCTCCA	120
AGI	AGACACGG	TTCTGAAGCT	ATTCAGAGAT	ATCAATCCTG	ACCTCTTTGT	GTTTGCAGAG	180
AT:	PANCGGAN	TGTACAACTC	TCCTTTCTTC	ATGACGAGGT	TCCGAGAAGC	GCTTTTTCAT	240
TAC	CTCTTCAC	TCTTTGACAT	GTTTGACACC	ACAATACACG	CAGAGGATGA	GTACAAAAAC	300
A GC	STCACTGT	TGGAGAGAGA	GTTACTTGTG	AGAGACGCGA	TGAGCGTGAT	TTCCTGCGAG	360
GG1	rgcagagc	GGTTTGCGAG	GCCTGAAACC	TACAAGCAAT	GGCGAGTTAG	GATTTTGAGA	420
GCC	CGGTTTA	AGCCAGCAAC	TATTAGCAAA	CAGATCATGA	AGGAGGCTAA	GGAAATTGTG	480
λGG	CAAACGTT	ACCATAGAGA	TTTTGTGATC	GATAGCGATA	ACAATTGGAT	GCTTCAAGGA	540
TGG	AAAGGAA	GAGTCATCTA	TGCTTTTTCT	TGCTGGAAAC	CTGCTGAGAA	GTTCACAAAC	600
דגג	*AATTTAA	ACATCTGAAA	AATGTTACTT	CTCAATTACA	TCATTTTTGT	TTCCCAATGG	660
TTI	TGTAGAA	TATGTTTGAT	CCCGTGAGTG	GATGCAACTC	TTTTTTCCTG	CAAGTACATA	720
TTG	TATTCAA	ATCCTTGTGG	AAATGATAAA	TTGTTTAATC	AAAAAAAAA	AAAAA	775

(2) INFORMATION FOR SEQ ID NO:50:

- (i) SEQUENCE CHARACTERISTICS:
 - (A) LENGTH: 206 amino acids
 - (B) TYPE: amino acid
 - (C) STRANDEDNESS: single
 - (D) TOPOLOGY: unknown

(ii) MOLECULE TYPE: peptide

(x1) SEQUENCE DESCRIPTION: SEQ ID NO:50:

Lys Lys Trp Glu Thr Ile Thr Leu Asp Glu Leu Met Ile Asn Pro Gly
1 10 15

Glu Thr Thr Val Val Asn Cys Ile His Arg Leu Gln Tyr Thr Pro Asp 20 25 30

Glu Thr Val Ser Leu Asp Ser Pro Arg Asp Thr Val Leu Lys Leu Phe 35 40 45

Arg Asp Ile Asn Pro Asp Leu Phe Val Phe Ala Glu Ile Asn Gly Met 50

Tyr Asn Ser Pro Phe Phe Met Thr Arg Phe Arg Glu Ala Leu Phe His 65 70 75 80

Tyr S r Ser Leu Phe Asp Met Phe Asp Thr Thr Ile His Ala Glu Asp 85 90 95

Glu Tyr Lys Asn Arg Ser Leu Leu Glu Arg Glu Leu Leu Val Arg Asp

			100					105					110		
Ala	Het	Ser 115	Val	Ile	Ser	Cys	Glu 120	Gly	Ala	Glu	λrg	Phe 125	Ala	Arg	Pro
G lu	Thr 130	Tyr	Lys	Gln	Trp	Arg 135	Val	Arg	Ile	Leu	Arg 140	Ala	Gly	Phe	Lys
Pro 145	Ala	Thr	Ile	Ser	Lys 150	Gln	Ile	Het	Lys	Glu 155	Ala	Lys	Glu	Ile	Val 160
Arg	Lys	Arg	Tyr	His 165	Arg	увь	Phe	Val	Ile 170	Хвр	Ser	λsp	λsn	As n 175	Trp
Met	Leu	Gln	Gly 180	Trp	Lys	Gly	Arg	Val 185	Ile	Tyr	Ala	Phe	Ser 190	Cys	Trp
Lys	Pro	Ala	Glu	Lys	Phe	Thr	Aen	Aen	λsn	Leu	Asn	Ile	Xaa		

200

205

(2) INFORMATION FOR SEQ ID NO:51:

195

- (i) SEQUENCE CHARACTERISTICS:
 - (A) LENGTH: 548 base pairs
 (B) TYPE: nucleic acid
 (C) STRANDEDNESS: unknown
 (D) TOPOLOGY: unknown
- (ii) MOLECULE TYPE: cDNA

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:51:

AATCGCTTGA	ACCGAATTTG	GATCGAGATT	CGAAAGAAAG	GCTGAGAGTG	GAGAGAGTGC	60
TGTTCGGTAG	GAGGATTATG	GATTTGGTCC	GATCAGATGA	TGATAATAAT	AAACCGGGAA	120
CCCGGTTTGG	GTTAATGGAG	GAGAAAGAAC	AATGGAGAGT	GTTGATGGAG	AAAGCTGGAT	180
TTGAGCCGGT	TAAACCGAGT	AATTACGCGG	TTAGCCAAGC	GAAGCTGCTA	CTATGGAACT	240
ACARTTATAG	TACATTGTAT	TCACTTGTTG	AATCGGAGCC	AGGTTTCATC	TCCTTGGCTT	300
GGAACAATGT	GCCTCTCCTC	ACCGTTTCCT	CTTGGCGTTG	ACTACTTGGT	CCGATAAGTT	360
AATCTAGTAT	TTTGAGTTAG	CTTTTAGAAT	TGAATTGTTT	GCGGTTAGAT	TTGGATGTTT	420
AATTAGTCTC	TAGCCTATTC	TCTTACTCTT	TTTTGTCTAG	TGCTTGGAGT	GATGATGGTT	480
TGTCGTTTAT	GTTCATTTGT	AATATATATT	GTATGTAACA	TTTGACTAAA	********	540
AAAAAAA						548

(2) INFORMATION FOR SEQ ID NO:52:

- (i) SEQUENCE CHARACTERISTICS:
 - (A) LENGTH: 113 amino acids
 (B) TYPE: amino acid
 (C) STRANDEDNESS: single
 (D) TOPOLOGY: unknown
- (ii) MOLECULE TYPE: peptide

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:52:

Ser Leu Glu Pr Asn Leu Asp Arg Asp Ser Lys Glu Arg Leu Arg Val 1 5 10 15

Glu Arg Val Leu Phe ly Arg Arg Ile Net Asp Leu Val Arg Ser Asp 20 25 30

Asp Asp Asn Asn Lys Pro Gly Thr Arg Phe Gly Leu Met Glu Glu Lys 35 40 45

Glu Gln Trp Arg Val Leu Met Glu Lys Ala Gly Phe Glu Pro Val Lys
50 55 60

Pro Ser Asn Tyr Ala Val Ser Gln Ala Lys Leu Leu Leu Trp Asn Tyr 65 70 75 80

Asn Tyr Ser Thr Leu Tyr Ser Leu Val Glu Ser Glu Pro Gly Phe Ile 85 90 95

Ser Leu Ala Trp Asn Asn Val Pro Leu Leu Thr Val Ser Ser Trp Arg 100 105 110

Xaa

(2) INFORMATION FOR SEQ ID NO:53:

- (i) SEQUENCE CHARACTERISTICS:
 - (A) LENGTH: 1093 base pairs
 - (B) TYPE: nucleic acid
 - (C) STRANDEDNESS: unknown
 - (D) TOPOLOGY: unknown

(ii) MOLECULE TYPE: CDNA

(x1) SEQUENCE DESCRIPTION: SEQ ID NO:53:

GCGAATGTTG AGATCTTGGA AGCAATAGCT GGGGAAACCA GAGTCCACAT TATCGATTTT 60 CAGATTGCAC AGGGATCACA ATACATGTTT TTGATTCAGG AGCTTGCGAA ACGCCCTGGT 120 GGGCCGCCGT TGCTGCGTGT GACGGGTGTG GATGATTCAC AGTCCACCTA TGCTCGTGGG 180 GGAGGACTCA GCTTGGTAGG TGAGAGGCTT GCAACTTTGG CGCAGTCATG TGGTGTCCCG 240 TTTGAGTTTC ACGATGCCAT CATGTCTGGG TGCAAGGTGC AGCGGGAACA TCTCGGGTTG 300 GAACCTGGCT TTGCTGTTGT TGTGAACTTC CCATATGTAT TACACCACAT GCCAGACGAG 360 AGCGTAAGTG TTGAAAAATA CAGAGACAGG CTGCTGCATC TGATCAAGAG CCTCTCCCCA 420 AAACTGGTTA CTCTAGTAGA GCAAGAATCC AACACAAACA CCTCGCCATT GGTGTCACGG 480 TTTGTGGAAA CACTGGATTA CTACACAGCG ATGTTTGAGT CGATAGATGC AGCACGGCCA 540 CGGGATGATA AGCAGAGAAT CAGCGCAGAA CAACACTGTG TAGCAAGAGA CATAGTGAAC 600 ATGATAGCAT GTGAGGAGTC AGAGAGAGTA GAGAGACACG AGGTACTGGG GAAATGGAGG GTCAGAATGA TGATGGCTGG GTTCACGGGT TGGCCGGTCA GCACATCTGC AGCGTTTGCA 720 CGAGTGAGA TGCTGAAAGC TTATGACAAA AACTACAAAC TGGGAGGCCA TGAAGGAGCG 780

CTCTACCTCT TCTGGAAGAG ACGACCCATG GCTACATGTT CCGTGTGGAA GCCAAACCCA 840

AACTATATTG GGTAAGTTAT AGTGATGATG TTACTTGAG TGGATAAAGA AGAGCACAAC 900

AAAAACACAT CTGTCGCTGT AAATTTTTTA GGATGTGCAA TGATGTTTTA AGTTGTAACA 960

CAACCTAAGT TATATATGTA TACAAACCAA ACCTGGTGGT TGTTTTTCTC TTGTAAATTG 1020

TCATGTGGTT GTGGGTGGGA AGCTAGTAAT GAAATATAAC CAAAACATTG ATTAGGTCAA 1080

AAAAAAAAAA AAA

(2) INFORMATION FOR SEQ ID NO:54:

- (1) SEQUENCE CHARACTERISTICS:
 - (A) LENGTH: 285 amino acide
 - (B) TYPE: amino acid
 - (C) STRANDEDNESS: single
 - (D) TOPOLOGY: unknown

(ii) MOLECULE TYPE: peptide

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:54:

Ala Asn Val Glu Ile Leu Glu Ala Ile Ala Gly Glu Thr Arg Val His 1 10 15

Ile Ile Asp Phe Gln Ile Ala Gln Gly Ser Gln Tyr Met Phe Leu Ile 20 25 30

Gln Glu Leu Ala Lys Arg Pro Gly Gly Pro Pro Leu Leu Arg Val Thr 35 40 45

Gly Val Asp Asp Ser Gln Ser Thr Tyr Ala Arg Gly Gly Gly Leu Ser 50 60

Leu Val Gly Glu Arg Leu Ala Thr Leu Ala Gln Ser Cys Gly Val Pro 65 70 75 80

Phe Glu Phe His Asp Ala Ile Met Ser Gly Cys Lys Val Gln Arg Glu 85 90 95

His Leu Gly Leu Glu Pro Gly Phe Ala Val Val Val Asn Phe Pro Tyr 100 105 110

Val Leu His His Met Pro Asp Glu Ser Val Ser Val Glu Lys Tyr Arg 115 120 125

Asp Arg Leu Leu His Leu Ile Lys Ser Leu Ser Pro Lys Leu Val Thr 130 135 140

Leu Val Glu Gln Glu Ser Asn Thr Asn Thr Ser Pro Leu Val Ser Arg 145 150 155 160

Phe Val Glu Thr Leu Asp Tyr Tyr Thr Ala Met Phe Glu Ser Ile Asp 165 170 175

Ala Ala Arg Pro Arg Asp Asp Lys Gln Arg Ile Ser Ala Glu Gln His 180 185 190

Cys Val Ala Arg Asp Ile Val Asn Met Ile Ala Cys Glu Glu Ser Glu 195 200 205

Arg Val Glu Arg His Glu Val Leu Gly Lys Trp Arg Val Arg Het Het

210 215 220

Met Ala Gly Phe Thr Gly Trp Pr Val Ser Thr Ser Ala Ala Phe Ala 225 230 235 240

Ala Ser Glu Het Leu Lys Ala Tyr Asp Lys Asn Tyr Lys Leu Gly Gly
245 250 255

His Glu Gly Ala Leu Tyr Leu Phe Trp Lys Arg Arg Pro Het Ala Thr 260 265 270

Cys Ser Val Trp Lys Pro Asn Pro Asn Tyr Ile Gly Xaa 275 280 285

(2) INFORMATION FOR SEQ ID NO:55:

- (1) SEQUENCE CHARACTERISTICS:
 - (A) LENGTH: 1928 base pairs
 - (B) TYPE: nucleic acid
 - (C) STRANDEDNESS: unknown
 - (D) TOPOLOGY: unknown

(ii) MOLECULE TYPE: CDNA

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:55:

ARAGACTITA GCAGATTITC AAGCGGCTCA GAACATCAAC AACAACAACA ACAACAACG 60 TITTATAGTC AAGCAGCTCT CAACGCTTTT CTTTCAAGGT CTGTGAAGCC TCGAAATTAT 120 CAGAATTTTC AATCTCCGTC GGCCGATGAT TGATCTCACG TCGGTGAATG ATATGAGTTT 180 GTTTGGTGGT TCTGGTTCAT CTCAGCGTTA CGGTTTACCG GTTCCCAGGT CTCAGACGCA 240 ACAGCAACAA TCGGATTACG GTTTATTTGG TGGGATCCGA ATGGGAATCG GGTCGGGTAT 300 TANTANTTAT CCARCATTAN CCGGCGTTCC GTGTATTGAN CCGGTTCANN ACCGGGTTCA 360 TGANTCGGAG ANCATGTTGA ATAGTTTANG AGAGCTTGAG ANACAGCTTT TAGATGATGA 420 CGATGAGAGT GGTGGTGATG ATGACGTGTC AGTTATAACA AATTCAAATT CCGATTGGAT 480 TCANANTCTC GTGACTCCGA ACCCGAACCC GAACCCGGTT TTGTCTTTTT CACCGAGCTC 540 TTCTTCTTCG TCTTCTTCGC CTTCTACAGC TTCGACGACG ACATCGGTAT GTTCTAGGCA 600 AACGGTTATG GAAATCGCGA CGGCGATCGC GGAAGGGAAA ACAGAGATAG CGACGGAGAT 660 TTTGGCGCGT GTTTCTCAAA CGCCTAATCT TGAGAGGAAT TCAGAGGAGA AGCTTGTTGA 720 TTTCATGGTG GCTGCGCTTC GATCGAGGAT AGCTTCTCCA GTGACGGAAT TGTATGGGAA 780 GAGCATTTA ATCTCGACTC AATTGCTCTA CGAGCTCTCT CCTTGTTTCA AACTCGGTTT **B40** GAGGCCGCG AATCTCGCCA TTCTCGACGC CGCCGATAAC AACGACGGTG GAATGATGAT 900 ACCGCACGIT ATCGATITCG ATATCGGAGA AGGTGGACAA TACGITAACC TICTCCGTAC 960 ATTATCCACG CGCCGGAATG GTAAAAGTCA GAGTCAGAAT TCTCCGGTGG TTAAGATCAC 1020 CGCCGTGGCG AACAACGTTT ACGGATGTTT AGTCGATGAC GGTGGAGAAG A AGGTTAAA 1080 AGCCGTCGGA GATTTGTTGA GCCAACTCGG TGATCGACTC GGTATCTCCG TAAGTTTCAA 1140

CGTGGTGACG AGTTTACGAC TCGGTGATCT AATCGTGAA TCTCTCGGGT GTGATCCCGA 1200 CGAGACTITG GCTGTGAACT TAGCTTTCAA GCTTTATCGT GTTCCCGACG AAAGCGTATG 1260 CACGGAGAAT CCAAGAGACG AACTTCTCCG CGCGTGAAG GGACTTAAAC CGCGCGTGGT 1320 TACTOTAGTG GAGCAAGAAA TGAATTCGAA TACGGCGCCC TTTTTAGGGA GAGTGAGTGA 1380 GTCATGCGCG TGTTACGGTG CGTTGCTTGA GTCGGTCGAG TCTACGGTTC CTAGTACGAA 1440 TTCCGACCGT GCCAAAGTTG AGGAAGGAAT TGGCCGGAAG CTAGTAAACG CGGTGGCGTG 1500 CGAAGGAATC GATCGTATAG AGCGGTGCGA GGTGTTCGGG AAATGGCGAA TGCGGATGAG 1560 CATGGCTGGG TTTGAGTTAA TGCCATTGAG TGAGAAGATA GCGGAGTCGA TGAAGAGTCG 1620 TGGAAACCGA GTCCACCCGG GCTTTACCGT TAAAGAAGAT AACGGAGGTG TGTGCTTTGG 1680 1740 TTGGATGGGA CGGGCACTCA CTGTCGCATC CGCTTGGCGT TAACTTCACA CACTCTTTTT 1800 TTTCTTCTTA TTATTACCAT ATTATTATTA ATTTTCGAGA TTATTCTGAT ATTATTATCA TTGTGATTTT CCGTTTCGAA AAGTGTAGGA ATCTTATGTA ACAAAGAAAA AAAAAAGACT 1860 TTTATGTTTT TCTAATAATA AAAGAAGAG TGATTGGGTT CAAAAAAAAA AAAAAAAAA 1920 1928 ΑΑΑΑΑΑΑ

(2) INFORMATION FOR SEQ ID NO:56:

- (i) SEQUENCE CHARACTERISTICS:
 - (A) LENGTH: 524 amino acids

 - (B) TYPE: amino acid (C) STRANDEDNESS: single
 - (D) TOPOLOGY: unknown
- (ii) MOLECULE TYPE: peptide

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:56:

Asp Leu Thr Ser Val Asn Asp Met Ser Leu Phe Gly Gly Ser Gly Ser 15

Ser Gln Arg Tyr Gly Leu Pro Val Pro Arg Ser Gln Thr Gln Gln Gln

Gln Ser Asp Tyr Gly Leu Phe Gly Gly Ile Arg Met Gly Ile Gly Ser

Gly Ile Asn Asn Tyr Pro Thr Leu Thr Gly Val Pro Cys Ile Glu Pro

Val Gin Asn Arg Val His Glu Ser Glu Asn Het Leu Asn Ser Leu Arg

Glu Leu Glu Lys Gln Leu Leu Asp Asp Asp Glu Ser Gly Gly Asp

Asp Asp Val Ser Val Ile Thr Asn Ser Asn Ser Asp Trp Ile Gln Asn

Leu Val Thr Pro Asn Pro Asn Pro Asn Pro Val Leu Ser Phe Ser Pr 120 115

Ser Ser Ser Ser S r Ser Ser Pro Ser Thr Ala Ser Thr Thr Ser Val Cys Ser Arg Gin Thr Val Het Glu Ile Ala Thr Ala Ile Ala Glu Gly Lys Thr Glu Ile Ala Thr Glu Ile Leu Ala Arg Val Ser Gln Thr Pro Asn Leu Glu Arg Asn Ser Glu Glu Lys Leu Val Asp Phe Met Val Ala Ala Leu Arg Ser Arg Ile Ala Ser Pro Val Thr Glu Leu Tyr 200 Gly Lye Glu His Leu Ile Ser Thr Gln Leu Leu Tyr Glu Leu Ser Pro 215 Cys Phe Lys Leu Gly Phe Glu Ala Ala Asn Leu Ala Ile Leu Asp Ala Ala Asp Asn Asn Asp Gly Gly Met Met Ile Pro His Val Ile Asp Phe Asp Ile Gly Gly Gly Gln Tyr Val Asn Leu Leu Arg Thr Leu Ser Thr Arg Arg Asn Gly Lys Ser Gln Ser Gln Asn Ser Pro Val Val Lys Ile Thr Ala Val Ala Asn Asn Val Tyr Gly Cys Leu Val Asp Asp Gly Gly Glu Glu Arg Leu Lys Ala Val Gly Asp Leu Leu Ser Gln Leu Gly 305 310 315 320 Asp Arg Leu Gly Ile Ser Val Ser Phe Asn Val Val Thr Ser Leu Arg 325 Leu Gly Asp Leu Asn Arg Glu Ser Leu Gly Cys Asp Pro Asp Glu Thr Leu Ala Val Asn Leu Ala Phe Lys Leu Tyr Arg Val Pro Asp Glu Ser Val Cys Thr Glu Asn Pro Arg Asp Glu Leu Leu Arg Arg Val Lys Gly Leu Lys Pro Arg Val Val Thr Leu Val Glu Glu Glu Het Asn Ser Asn Thr Ala Pro Phe Leu Gly Arg Val Ser Glu Ser Cys Ala Cys Tyr Gly Ala Leu Leu Glu Ser Val Glu Ser Thr Val Pro Ser Thr Asn Ser Asp Arg Ala Lys Val Glu Glu Gly Ile Gly Arg Lys Leu Val Asn Ala Val 435 440 445 Ala Cys Glu Gly Ile Asp Arg Ile Glu Arg Cys Glu Val Phe Gly Lys Trp Arg Met Arg Met Ser M t Ala Gly Phe lu Leu Met Pro Leu Ser Glu Lys Il Ala Glu Ser M t Lys Ser Arg Gly Asn Arg Val His Pr

> 485 490 495

Gly Phe Thr Val Lys Glu Asp Asn Gly Gly Val Cys Phe Gly Trp Met 500 505

Gly Arg Ala Leu Thr Val Ala Ser Ala Trp Arg Xaa 520 515

(2) INFORMATION FOR SEQ ID NO:57:

- (i) SEQUENCE CHARACTERISTICS:
 - (A) LENGTH: 2635 base pairs

 - (B) TYPE: nucleic acid (C) STRANDEDNESS: unknown (D) TOPOLOGY: unknown

(11) MOLECULE TYPE: CDNA

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:57:

TCTTACTCAA	GGTTCTTCTT	TGTCATCTTG	TTGCCGAATC	CACAAAGAGG	AGAATAAAGA	60
TTCGACCTTT	ATTAGATATT	AACGACTCTG	GATTTTTGGG	TTTTTGGAGT	TGGATCCACA	120
TGGGTTCTTA	TCCGGATGGA	TTCCCTGGAT	CCATGGACGA	GTTGGATTTC	AATAAGGACT	180
TTGATTTGCC	TCCCTCCTCA	AACCAAACCT	TAGGTTTAGC	TAATGGGTTC	TATTTAGATG	240
ACTTAGATTT	CTCATCCTTG	GATCCTCCAG	AGGCATATCC	CTCCCAGAAC	AACAACAACA	300
ACAACATCAA	CAACAAAGCT	GTAGCAGGAG	ATCTGTTATC	ATCTTCATCT	GATGACGCTG	360
ATTTCTCTGA	TTCTGTTTTG	AAGTATATAA	GCCAAGTTCT	TATGGAAGAG	GATATGGAAG	420
A AAGCCTTG	TATGTTTCAT	GATGCTTTGG	CTCTTCAAGC	TGCTGAGAAA	TCTCTCTATG	480
AGGCTCTTGG	TGAGAAAGAC	CCTTCTTCGT	CTTCTGCTTC	TTCTGTGGAT	CATCCTGAGA	540
ATTGGCTAG	TCATAGCCCT	GACGGTTCTT	GTTCAGGTGG	TGCTTTTAGT	GATTACGCTA	600
GCACCACTAC	CACTACTTCC	TCTGATTCTC	ACTGGAGTGT	TGATGGTTTG	GAGAATAGAC	660
CTTCTTGGTT	ACATACACCT	ATGCCGAGTA	ATTTTGTTTT	CCAGTCTACT	TCTAGGTCCA	720
ACAGTGTCAC	CGGTGGTGGT	CGTGGTGGTA	ATAGTGCGGT	TTACGGTTCA	GGTTTTGGCG	780
ATGATTTGGT	TTCGAATATC	TTTAAAGATG	ATGAATTGGC	TATGCAGTTC	AAGAAAGGGG	840
TTGAGGAAGC	TAGTAAGTTC	CTTCCTAAGT	CTTCTCAGCT	CTTTATTGAT	GTGGATAGTT	900
ACATCCCTAT	GAATTCTGGT	TCCAAGGAAA	ATGGTTCTGA	GGTTTTTGTT	AAGACGGAGA	960
AGAAAGATGA	GACAGAGCAT	CATCATCATC	ATAGCTATGC	ACCACCACCC	AACAGATTAA	1020
					AGAAGTAACA	1080
					AACATGTTCC	1140
					GAATCCGCTA	1200
					ACTTCTACTA	1260
					GTGTTATGTG	1320
ATONINAIN	" CAUTICIAN					

CACAAGCTGT	ATCAGTGGAT	ATCGTAGAA	CCGCCAACGI	TTAGCTAAGG	CAGATACGAG	1380
AGCATTCTTC	GCCTCTAGGC	AATGGTTCAG	AGCGGTTGGC	TCATTATTTI	GCAAATAGTC	1440
TTGAAGCACG	CTTAGCTGGG	ACCGGTACAC	AGATETACAC	CGCTTTATCT	TCGAAGAAAA	1500
CGTCTGCAGC	AGACATGTTG	AAGGCTTACC	AGACATACAT	GTCGGTCTGC	CCTTTCAAGA	1560
aagctgctat	CATATTTGCT	AACCACAGCA	TGATGCGTTT	CACTGCAAAC	GCCAACACGA	1620
TCCACATAAT	AGATTTCGGA	ATATCTTACG	GTTTTCAGTG	GCCTGCTCTG	ATTCATCGCC	1680
TCTCGCTCAG	CAGACCTGGT	GGTTCGCCTA	AGCTTCGAAT	TACCGGTNNN	инининини	1740
иииииииии	NNNNNNNNN	NNNGAGTTCA	GGAGACAGGT	CATCGCTTGG	CTCGATACTG	1800
TCAGCGACAC	AATGTTCCGT	TTGAGTACAA	CGCAATTGCT	CAGAAATGGG	GAAACGATCC	1860
aagtegaaga	CTTAAAGCTT	CGACAAGGAG	AGTATGTGGT	TGTGAACTCT	TIGTICCOTT	1920
TCAGGAACCT	TCTAGATGAG	ACCGTTCTGG	TAAACAGCCC	GAGAGATGCA	GTTTTGAAGC	1980
TGATAAGAAA	AATAAACCCG	AATGTCTTCA	TTCCAGCGAT	CTTAAGCGGG	AATTACAACG	2040
CGCCATTCTT	TGTCACGAGG	TTCAGAGAAG	CGTTGTTTCA	TTACTCGGCT	GTGTTTGATA	2100
TGTGTGACTC	GAAGCTAGCT	AGGGAAGACG	AGATGAGGCT	GATGTATGTG	TTTGAGTTTT	2160
atgggagaga	GATTGTGAAT	GTTGTGGCTT	CTGAAGGAAC	AGAGAGAGTG	GAGAGCCGAG	2220
AGACATATAA	GCAGTGGCAG	GCGAGACTGA	TCCGAGCCGG	ATTTAGACAG	CTTCCGCTTG	2280
AGAAGGAACT	GATGCAGAAT	CTGAAGTTGA	AAATCGAAAA	CGGGTACGAT	AAAAACTTCG	2340
atgitgatca	AAACGGTAAC	TGGTTACTTC	AAGGGTGGAA	AGGTAGAATC	GTGTATGCTT	2400
CATCTCTATG	GGTTCCTTCG	TCTTCATAGA	TGTTGTTTCT	TACGTTCTAA	GCGACTGGGA	2460
TTATGTAGG	GCTTTTCTGT	TGATAGTCTC	TCGCCAACAC	GAGTGGATTA	AGTTCAGAGT	2520
PAGGGTTCTT	Gaacactaga	Atgttgttat	ATTATGCTTG	TGACATAGCG	TGTGTAAGAG	2580
rgtageetaa	GAGATATAGT	actcattgca	TGATCTTTTG	CTATATGTTN	CATGT	2635

(2) INFORMATION FOR SEQ ID NO:58:

(i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 809 amino acids
 (B) TYPE: amino acid
 (C) STRANDEDNESS: single
 (D) TOPOLOGY: unknown

(ii) MOLECULE TYPE: peptide

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:58:

Leu Leu Lys Val Leu Leu Cys His Leu Val Ala Glu Ser Thr Lys Arg

Arg Ile Lys Ile Arg Pro Leu Leu Asp Ile Asn Asp Ser Gly Ph Leu

Gly Phe Trp Ser Trp Ile His Het Gly S r Tyr Pr Asp Gly Phe Pr 35 40 45

Gly Ser Net Asp Glu Leu Asp Ph Asn Lys Asp Phe Asp Leu Pro Pro Ser Ser Asn Gln Thr Leu Gly Leu Ala Asn Gly Ph Tyr Leu Asp Asp Leu Asp Phe Ser Ser Leu Asp Pro Pro Glu Ala Tyr Pro Ser Gln Asn Asn Asn Asn Asn Ile Asn Asn Lys Ala Val Ala Gly Asp Leu Leu 100 105 110 Ser Ser Ser Asp Asp Ala Asp Phe Ser Asp Ser Val Leu Lys Tyr 115 120 125 Ile Ser Gln Val Leu Het Glu Glu Asp Het Glu Glu Lys Pro Cys Het Phe His Asp Ala Leu Ala Leu Gln Ala Ala Glu Lys Ser Leu Tyr Glu Ala Leu Gly Glu Lys Asp Pro Ser Ser Ser Ser Ala Ser Ser Val Asp His Pro Glu Arg Leu Ala Ser His Ser Pro Asp Gly Ser Cys Ser Gly 185 Gly Ala Phe Ser Asp Tyr Ala Ser Thr Thr Thr Thr Thr Ser Ser Asp Ser His Trp Ser Val Asp Gly Leu Glu Asn Arg Pro Ser Trp Leu His Thr Pro Met Pro Ser Asn Phe Val Phe Gln Ser Thr Ser Arg Ser Asn Ser Val Thr Gly Gly Gly Gly Gly Gly Asn Ser Ala Val Tyr Gly Ser 255 Gly Phe Gly Asp Asp Leu Val Ser Asn Met Phe Lys Asp Asp Glu Leu 260 265 270 Ala Met Gln Phe Lys Lys Gly Val Glu Glu Ala Ser Lys Phe Leu Pro Lys Ser Ser Gln Leu Phe Ile Asp Val Asp Ser Tyr Ile Pro Met Asn 295 Ser Gly Ser Lys Glu Asn Gly Ser Glu Val Phe Val Lys Thr Glu Lys Lys Asp Glu Thr Glu His His His His His Ser Tyr Ala Pro Pro Pro Asn Arg Leu Thr Gly Lys Lys Ser His Trp Arg Asp Glu Asp Glu Asp Phe Val Glu Glu Arg Ser Asn Lys Gln Ser Ala Val Tyr Val Glu Glu 355 360 365 Ser Glu Leu Ser Glu Het Phe Asp Asn Het Phe Leu Cys Gly Pro Gly 380 Lys Pro Val Cys Ile Leu Asn Gln Asn Phe Pro Thr Glu Ser Ala Lys Val Val Thr Ala Gln Ser Asn Gly Ala Lys Ile Arg Gly Lys Lys Ser

405 410 415

Thr Ser Thr Ser His Ser Asn Asp Ser Lys Clu Thr Ala Asp Leu Arg Thr Leu Leu Val Leu Cys Ala Gln Ala Val Ser Val Asp Asp Arg Arg Thr Ala Asn Val Xaa Leu Arg Gln Ile Arg Glu His Ser Ser Pro Leu Gly Asn Gly Ser Glu Arg Leu Ala His Tyr Phe Ala Asn Ser Leu 470 475 Glu Ala Arg Leu Ala Gly Thr Gly Thr Gln Ile Tyr Thr Ala Leu Ser Ser Lys Lys Thr Ser Ala Ala Asp Met Leu Lys Ala Tyr Gln Thr Tyr Met Ser Val Cys Pro Phe Lys Lys Ala Ala Ile Ile Phe Ala Asn His Ser Net Met Arg Phe Thr Ala Asn Ala Asn Thr Ile His Ile Ile Asp Phe Gly Ile Ser Tyr Gly Phe Gln Trp Pro Ala Leu Ile His Arg Leu Ser Leu Ser Arg Pro Gly Gly Ser Pro Lys Leu Arg Ile Thr Gly Xaa 570 Val Ile Ala Trp Leu Asp Thr Val Ser Asp Thr Met Phe Arg Leu Ser Thr Thr Gln Leu Leu Arg Asn Gly Glu Thr Ile Gln Val Glu Asp Leu Lys Leu Arg Gln Gly Glu Tyr Val Val Val Asn Ser Leu Phe Arg Phe Arg Asn Leu Leu Asp Glu Thr Val Leu Val Asn Ser Pro Arg Asp Ala Val Leu Lys Leu Ile Arg Lys Ile Asn Pro Asn Val Phe Ile Pro Ala Ile Leu Ser Gly Asn Tyr Asn Ala Pro Phe Phe Val Thr Arg Phe Arg Glu Ala Leu Phe His Tyr Ser Ala Val Phe Asp Met Cys Asp Ser Lys Leu Ala Arg Glu Asp Glu Met Arg Leu Met Tyr Val Phe Glu Phe Tyr Gly Arg Glu Ile Val Asn Val Val Ala Ser Glu Gly Thr Glu Arg Val Glu Ser Arg Glu Thr Tyr Lys Gln Trp In Ala Arg Leu Ile Arg Ala Gly Phe Arg Gln Leu Pro Leu Glu Lys Glu Leu Met Gln Asn Leu Lys 760

Leu Lys Ile Glu Asn Gly Tyr Asp Lys Asn Ph Asp Val Asp Gln Asn 770. 775

Gly Asn Trp Leu L u Gln Gly Trp Lys Gly Arg Ile Val Tyr Ala Ser 785 790 795 800

Ser Leu Trp Val Pro Ser Ser Ser Xaa 805

(2) INFORMATION FOR SEQ ID NO:59:

- (i) SEQUENCE CHARACTERISTICS:
 - (A) LENGTH: 90 amino acids (B) TYPE: amino acid

 - (C) STRANDEDNESS: single
 - (D) TOPOLOGY: unknown
- (11) MOLECULE TYPE: peptide
- (xi) SEQUENCE DESCRIPTION: SEQ ID NO:59:

Gln Glu Ala Asp His Asn Lys Thr Gly Phe Leu Asp Arg Phe Thr Glu

Ala Leu Phe Tyr Tyr Ser Ala Val Phe Asp Ser Leu Asp Ala Ala Asn

Asn Asn Asn Asn Asn Asn Gln Arg Het Glu Ala Glu Tyr Leu Gln

Arg Glu Ile Cys Asp Ile Val Cys Gly Glu Gly Ala Ala Arg Xaa Glu

Arg His Glu Pro Leu Ser Arg Trp Arg Asp Arg Leu Thr Arg Ala Gly 75

Leu Ser Ala Val Pro Leu Gly Ser Asn Ala

(2) INFORMATION FOR SEQ ID NO: 60:

- (i) SEQUENCE CHARACTERISTICS:
 - (A) LENGTH: 199 base pairs (B) TYPE: nucleic acid (C) STRANDEDNESS: unknown

 - (D) TOPOLOGY: unknown
- (ii) MOLECULE TYPE: DNA (genomic)
- (vi) ORIGINAL SOURCE:
 - (A) ORGANISM: Daucus carota
- (xi) SEQUENCE DESCRIPTION: SEQ ID NO:60:

TCTGCAGACA ATTTTNAGGA G	GCCAATACC	ATGCTATTGG	AAATTTCAGA	ACTGTCCACA	60
ССТИНИНИИ ИНИНИНИНИ	нининини	NNNGTACTTC	TCAGAGGNAA	TGTCGGNNAG	120
ATTAGTTAGC TCCTGCTTAG	GAATCTATGC	TTCTCTTCCN	GCAACAGTGG	TGCCTCCTCA	180
TGGTCAGAAA TGGCCTCA					199

(2) INFORMATION FOR SEQ ID NO:61:

- (i) SEQUENCE CHARACTERISTICS:
 - (A) LENGTH: 66 amino acids
 - (B) TYPE: amino acid
 - (C) STRANDEDNESS: singl
 - (D) TOPOLOGY: unknown
- (ii) MOLECULE TYPE: peptide
- (vi) ORIGINAL SOURCE:
 - (A) ORGANISM: Daucus carota
- (xi) SEQUENCE DESCRIPTION: SEQ ID NO:61:

Ser Ala Asp Asn Phe Xaa Glu Ala Asn Thr Het Leu Leu Glu Ile Ser

20 25

Phe Ser Glu Xaa Net Ser Xaa Arg Leu Val Ser Ser Cys Leu Gly Ile

Tyr Ala Ser Leu Pro Ala Thr Val Val Pro Pro His Gly Gln Lys Val 55

Ala Ser 65

(2) INFORMATION FOR SEQ ID NO:62:

- (i) SEQUENCE CHARACTERISTICS:
 - (A) LENGTH: 321 base pairs

 - (B) TYPE: nucleic acid (C) STRANDEDNESS: unknown
 - (D) TOPOLOGY: unknown
- (ii) MOLECULE TYPE: DNA (genomic)
- (vi) ORIGINAL SOURCE:
 - (A) ORGANISM: Glycine max
- (xi) SEQUENCE DESCRIPTION: SEQ ID NO:62:

TCAACTGAGA	ATCTAGAAGA	TGCCAACAAG	ATGCTTCTGG	AGATTTCTCA	GTTATCAACA	60
CCGTTCNNCA	CTTCAGCACA	GCGTGTGGCA	GCATATTTCT	CAGAAGCCAT	ATCAGCAAGG	120
TTGGTGAGTT	CATGTCTAGG	GATATACGCA	ACTTTGCCAC	ACACACACCA	AAGCCACAAG	180
GTAGCTTCAG	CTTTTCAAGT	GTTCAATGGT	ATTAGTCCTT	TAGTGGAGTT	CTCACACTTC	240
ACAGCAAACC	AAGCAATTCA	AGAAGCCTTC	GAAAGAGAAG	AGAGGGTGCA	CATCATAGAT	300
CTTGATATAA	TGCAAGGGTT	G				321

- (2) INFORMATION FOR SEQ ID NO:63:
 - (1) SEQUENCE CHARACTERISTICS:
 - (A) LENGTH: 107 amino acids
 - (B) TYPE: amino acid
 - (C) STRANDEDNESS: single

		(D)	TOI	OLO	ZY: ι	ınkno	wn										
	(ii)	HOLI	CULI	TYI	PE: 1	epti	de										
	(AŢ)		INAI ORG				ine s	nax									
	(xi)	SEQ	JENCI	E DE	SCRII	TIO	1: 81	BQ II	NO:	63:							
	Ser 1	Thr	Glu	Asn	Leu 5	Glu	Asp	Ala	Asn	Lys 10	Net	Leu	Leu	Glu	Ile 15	Ser	
	Gln	Leu	Ser	Thr 20	Pro	Phe	Xaa	Thr	Ser 25	Ala	Gln	Arg	Val	Ala 30	Ala	Tyr	
	Phe	Ser	Glu 35	Ala	Ile	Ser	Ala	Arg 40	Leu	Val	Ser	Ser	Cys 45	Leu	Gly	Ile	
	Tyr	Ala 50	Thr	Leu	Pro	His	Thr 55	His	Gln	Ser	His	Lys 60	Val	Ala	Ser	Ala	
	Phe 65	Gln	Val	Phe	Asn	Gly 70	Ile	Ser	Pro	Leu	Val 75	Glu	Phe	Ser	His	Phe 80	
	Thr	Ala	Asn	Gln	Ala 85	Ile	Gln	Glu	Ala	Phe 90	Glu	Arg	Glu	Glu	Arg 95	Val	
	Hie	Ile	Ile	Asp 100		Asp	Ile	Het	Gln 105	Gly	Leu						
(2)	INFO	RMAT	ION :	POR	SEQ	ID N	0:64	:									
	(i)	(A (B (C	UENC) LE) TY) ST	ngth Pe: Rand	: 19 nucl EDNE	5 ba eic ss:	se p acid unkn	airs									
	(ii)	MOL	ECUL	E TY	PE:	DNA	(gen	omic)								
	(vi)		GINA) OR				a										
	(xi)	SEQ	UENC	E DE	SCRI	PTIO	N: S	EQ I	D NO	:64:							
TCT	GCAGA	CA A	CTTI	GAAG	A AG	CCAA	TACA	ATA	CTGC	CTC	agat	CYCY	GA A	CTCT	CCAC	C	60
ccc	TATNO	ca a	CTCG	GTGC	A AC	gagi	GCT	. ecc	TATE	иии	MNNN	MNMN	inn n	INNNN	INNNN	N	120
HNN	NNNN	INN N	INTGO	:ATAC	G AA	TGTA	TTCI	r cc1	CTCC	CTC	CTAT	TCAC	AT C	TCCC	AGAG	C	180
CAG	וגגגג	TG 1	rgaat	•													195

- (2) INFORMATION FOR SEQ ID NO:65:
 - (i) SEQUENCE CHARACTERISTICS:

 (A) LENGTH: 65 amino acide

 (B) TYPE: amino acid

 (C) STRANDEDNESS: single

 (D) TOPOLOGY: unknown
 - (ii) MOLECULE TYPE: peptide

(vi) ORIGINAL SOURCE: (A) ORGANISM: Picea

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:65:

Ser Ala Asp Asn Phe Glu Glu Ala Asn Thr Ile Leu Pro Gln Ile Thr 1 5 10 15

Glu Leu Ser Thr Pro Tyr Xaa Asn Ser Val Gln Arg Val Ala Ala Tyr 20 25 30

Tyr Ser Pro Leu Pro Pro Ile His Met Ser Gln Ser Gln Lys Ile Val 50 55 60

Asn 65

(2) INFORMATION FOR SEQ ID NO:66:

- (1) SEQUENCE CHARACTERISTICS:
 - (A) LENGTH: 2151 base pairs
 - (B) TYPE: nucleic acid
 - (C) STRANDEDNESS: unknown
 - (D) TOPOLOGY: unknown
- (ii) MOLECULE TYPE: CDNA

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:66:

GATATCAGCA TCATCAATTT TAAATGTAAG TTGGCAAAAG ATCATGAGGG TTCTCATAGT 60 AATTTGGCCA CAAGGTATGA CACTGTCTCA ATTGAGCAAT CTAGTAGAGA AACTGATCCA 120 TCATATATTG CTCATATTGA AAGTGAAAAA GATATGCTCA AGAACCTAGT AGAGAAGCTA 180 AAAATTGAAA AATCTAGCTC TACTAGAAAA ATATGATAGG TTGCCTGTTT CTCATGAAAA 240 TTTATTAGAT AATCATATCA TEGETAGATE TEGETEATGA GETTETTETT GETAGTTTAG 300 ATTCCTGTGG GCATTCATCT CTTTTAGATG CACTAACATG ATAGGAAGTT TCTAATCTGG 360 TGCTTCACAA TTCTGGTGAT TCATGCTTCC TTCATTGCAA TTGATATTGA TGCTTGATTC 420 ATGCTTCAGT CACTTTGTGC GTTTAATTGG TATTGTATGT ATCACTAGAT TGTAGGGTGT 480 CTGCAACTAG TGTTTCACCA TGTGGTTTTT TAGTATCATT CGTATTAGTT TCTAACTTTC 540 TATTGATATA TTAAAGTGAT AACTAGTTTT AGAAATATTC TCTTGTGCCA TTAATGCTAC 600 AACTTGTTTT TAGCGTGTAC GTTAGCATTA TAATATTTCC TTATTATGAA AGCGGAAGAG 660 AAACGCGCCC AACCAGAGCA TCCACGTCGT CTCATTCAC CTTCATCGTT GGATCATAGA 720 TGAGCGGTCC ACGGTGAACT CCGTTTGCCT GCAAAACCAC GTCCTCTACG CGCTGTTAAG 780 TAGCTTCTAG AAACATCACG ATGTGTCCCG TCCATTCCTT TAGGAGGAGC CGGATCCGGC 840 GCCGCAGTCG CCCAAGGTCC CGACCGCCGC GGCCTCGGCC GCCGCCGCCA AGGAGCGGAA 900

GGAGGTGCAG CGGCGGAAGC AGCGCGACGA GGAGGGCCTC CACCTGCTGA GTGCTGACGC 960 TGCTGCTGCA GTGCGCGGAG CCGTGAACG CGGACAACCT CGACGACGCG CACCAGACGC 1020 TGCTGGAGAT CGCGGAGCTG GCCACGCCGT TCGGCACCTC ACCCAGCGC GTGGCCGCCT 1080 ACTTCGCGGA GGCCATGTCG GCGCGCGTCG TCAGCTCCTG CCTAGGCCTG TACGCGCCGC 1140 TGCCGCCGGG CTCCCCCGCC GCGGCGCGCCC TCCACGGCCG CGTGGCCGCC GCGTTCCAGG 1200 TGTTCAACGG CATCAGCCCC TTCGTCAAGT TCTCGCACTT CACCGCCAAC CAGGCCATCC 1260 AGGAGGCGTT CGAGCGGGAG GAGCGTGTGC ACATCATCGA CCTCGACATC ATGCAGGGGC 1320 TECAGTEGEC GEGECTETTE CACATECTTE TETECEGECE CGGGGGCCG CCCAGGGTCA 1380 GGCTCACCGG CCTGGGGGCG TCCATGGACG CGCTCGAGGC GACGGGGAAG CGCCTCTCCG 1440 ACTTCGCCGA CACGCTCGGC CTGCCCTTCG AGTTCTGCGC CGTCGCCGAG AAGGCCGGCA 1500 ACGITGACCC GCAGAAGCTG GGCGTCACGC GGCGGGAGGC CGTCGCCGTC CACTGGCCGC 1560 ACCACTOGOT TTACGACGTC ATCGGCTCCG ACTCCAACAC GCTCTGGCTC ATCCAAAGGT 1620 1680 CCTCCATTTT CCTTCTCCC CTTTCTTCCA TGTCAAATCT TGATGCAATC ATGACCACTT TTCAGCTGCT GACATTGGAT AATGTGAGCT TTACGGCAAG CATCAAGTCG TGGTAGTACA 1740 1800 TECATTACAG CTATTECTAA AATATTETTE GGAGGTTTEE TGCTCATAGT AAAAAAAAAT CGCGTTTTGA AGCTCAAAAG GCGATTTCTT CCGAGGTTTG CTGTTGAGCG CTATTTTGGA 1860 1920 AACCCCATTT TCTCAATTGA TTTTTATTTT TTAAAGAAAA ATTAGTTCAT TTTTCTCTTG TGAAATGGAG TCCCAAACTA ACCCTAATAT TAAAAAAAAC GCGCTTTGGA GCTCAAAACG 1980 CTCGTTGTTA TGACCAACCA GCTTTATAGG TTTAAAAAGG TTGAATCTTG ACAATGCTTT 2040 TGARAGGTT GARTCTTGAC ARTGCTTTTG AGRTGATACT GTAGTGTAGT CTGTAGTGGA 2100 GCATCCTCCA TGGTCTTTGG TGATCGAGAA TTCCTGCAGC CCGGGGGATC C 2151

(2) INFORMATION FOR SEQ ID NO:67:

- (1) SEQUENCE CHARACTERISTICS:
 - (A) LENGTH: 716 amino acids

 - (B) TYPE: amino acid (C) STRANDEDNESS: single
 - (D) TOPOLOGY: unknown
- (11) MOLECULE TYPE: peptide
- (xi) SEQUENCE DESCRIPTION: SEQ ID NO: 67:
- Tyr Gln His His Gln Phe Xaa Met Xaa Val Gly Lys Arg Ser Xaa Gly
- Phe Ser Xaa Xaa Phe Gly His Lys Val Xaa His Cys Leu Asn Xaa Ala
- Ile Xaa Xaa Arg Asn Xaa Ser Ile Ile Tyr Cys Ser Tyr Xaa Lys Xaa
- Lys Arg Tyr Ala Gln Glu Pro Ser Arg Glu Ala Lys Asn Xaa Lys Ile

50 55 60

Xaa Leu Tyr Xaa Lys Asn Het Ile Gly Cys Leu Ph Leu Het Lys Il Tyr Xaa Ile Ile Ser Trp Leu Asp Val Ala His Glu Val Val Leu Ala Ser Leu Asp Ser Cys Gly His Ser Ser Leu Leu Asp Ala Leu Thr Xaa Xaa Glu Val Ser Asn Leu Val Leu His Asn Ser Gly Asp Ser Cys Phe Leu His Cys Asn Xaa Tyr Xaa Cys Leu Ile His Ala Ser Val Thr 130 135 140 Leu Cys Val Xaa Leu Val Leu Tyr Val Ser Leu Asp Cys Arg Val Ser Ala Thr Ser Val Ser Pro Cys Gly Phe Leu Val Ser Phe Val Leu Val Ser Asn Phe Leu Leu Ile Tyr Xaa Ser Asp Asn Xaa Phe Xaa Lys Tyr Ser Leu Val Pro Leu Met Leu Gln Leu Val Phe Ser Val Tyr Val Ser Ile Ile Phe Pro Tyr Tyr Glu Ser Gly Arg Glu Thr Arg Pro Thr Arg Ala Ser Thr Ser Ser His Phe Thr Phe Ile Val Gly Ser Xaa Met Ser Gly Pro Arg Xaa Thr Pro Phe Ala Cys Lys Thr Thr Ser Ser Thr Arg Cys Xaa Val Ala Ser Arg Asn Ile Thr Met Cys Pro Val His Ser Phe Arg Arg Ser Arg Ile Arg Arg Arg Ser Arg Pro Arg Ser Arg Pro Pro Arg Pro Arg Pro Pro Pro Arg Ser Gly Arg Arg Cys Ser Gly Gly Ser Ser Ala Thr Arg Arg Ala Ser Thr Cys Xaa Val Leu Thr Leu Leu Leu Gln Cys Ala Glu Ala Val Asn Ala Asp Asn Leu Asp Asp Ala His Gln Thr Leu Leu Glu Ile Ala Glu Leu Ala Thr Pro Phe Gly Thr Ser Thr Gln Arg Val Ala Ala Tyr Phe Ala Glu Ala Het Ser Ala Arg Val Val Ser Ser Cys Leu Gly Leu Tyr Ala Pro Leu Pro Pro Gly Ser 370 380 Pro Ala Ala Arg Leu His Gly Arg Val Ala Ala Ala Phe Gln Val Ph Asn Gly Ile S r Pr Phe Val Lys Phe S r His Phe Thr Ala Asn

Gin Ala Ile Gin Glu Ala Phe Glu Arg Glu Glu Arg Val His Ile Ile Asp Leu Asp Ile M t In Gly Leu Gln Trp Pro ly Leu Phe His Il Leu Val Ser Arg Pro Gly Gly Pro Pro Arg Val Arg Leu Thr Gly Leu 450 455 Gly Ala Ser Net Asp Ala Leu Glu Ala Thr Gly Lys Arg Leu Ser Asp 465 470 475 Phe Ala Asp Thr Leu Gly Leu Pro Phe Glu Phe Cys Ala Val Ala Glu Lys Ala Gly Asn Val Asp Pro Gln Lys Leu Gly Val Thr Arg Arg Glu Ala Val Ala Val His Trp Pro His His Ser Leu Tyr Asp Val Ile Gly Ser Asp Ser Asn Thr Leu Trp Leu Ile Gln Arg Ser Ser Ile Phe Leu Lau Cys Leu Ser Ser Met Ser Asn Leu Asp Ala Ile Met Thr Thr Phe Gln Leu Leu Thr Leu Asp Asn Val Ser Phe Thr Ala Ser Ile Lys Ser 570 Trp Xaa Tyr Ile His Tyr Ser Tyr Phe Xaa Asn Ile Leu Arg Arg Phe Pro Ala His Ser Lys Lys Ser Arg Phe Glu Ala Gln Lys Ala Ile 600 Ser Ser Glu Val Cys Cys Xaa Ala Leu Phe Trp Lys Pro His Phe Leu Asn Xaa Phe Leu Phe Phe Lys Glu Lys Leu Val His Phe Ser Leu Val Lys Trp Ser Pro Lys Leu Thr Leu Ile Leu Lys Lys Thr Arg Phe Gly Ala Gln Asn Ala Arg Cys Tyr Asp Gln Pro Ala Leu Xaa Val Xaa Lys Gly Xaa Ile Leu Thr Met Leu Leu Lys Arg Leu Asn Leu Asp Asn Ala Phe Glu Met Ile Leu Xaa Cys Ser Leu Xaa Trp Ser Ile Leu His Gly Leu Trp Xaa Ser Arg Ile Pro Ala Ala Arg Gly Ile

WHAT IS CLAIMED IS:

 An isolated nucl ic acid molecule comprising a nucleotide sequence that encodes a SCARECROW protein
 containing an amino acid sequence substantially similar to the sequence of MOTIF III (VHIID) of Arabidopsis SCR protein shown in FIGS. 13A-F.

- 2. An isolated nucleic acid molecule comprising a nucleotide sequence that (a) encodes a scarecrow protein having the amino acid sequence shown of any one of SEQ ID NO:2, SEQ ID NO:19, SEQ ID NO:21, SEQ ID NO:23, SEQ ID NO:34, SEQ ID NO:35, SEQ ID NO:36, SEQ ID NO:37, SEQ ID NO:41, SEQ ID NO:42, SEQ ID NO:43, SEQ ID NO:44, SEQ ID NO:46, SEQ ID NO:48, SEQ ID NO:50, SEQ ID NO:51, SEQ ID NO:52, SEQ ID NO:54, SEQ ID NO:56, SEQ ID NO:58, SEQ ID NO:59, SEQ ID NO:61, SEQ ID NO:63, SEQ ID NO:65 or SEQ ID NO:67; or (b) is the complement of the nucleotide sequence of (a).
- 20 3. An isolated nucleic acid molecule comprising a nucleotide sequence that hybridizes to the nucleic acid of Claim 2 and encodes a naturally occurring SCR gene product.
- 4. A nucleic acid molecule comprising a nucleotide sequence 25 that (a) encodes a SCR protein lacking one to four of the following motifs delineated in FIGS. 13A-P: MOTIF I, MOTIF II, MOTIF IV, MOTIF V, or MOTIF VI; or (b) is the complement of the nucleotide sequence of (a).
- 30 5. A nucleic acid molecule comprising a nucleotide sequence that (a) encodes a polypeptide corresponding to MOTIF I, MOTIF II, MOTIF IV, MOTIF V or MOTIF VI of the SCARECROW protein delineated in FIGS. 13A-F; or (b) is the complement of the nucleotide sequence of (a).
 - 6. The isolated nucleic acid molecule of Claim 1 comprising the nucl ic acid s quence of any on of SEQ ID NO:1, SEQ ID

NO:18, SEQ ID NO:20, SEQ ID NO:22, SEQ ID NO:45, SEQ ID NO:47, SEQ ID NO:49, SEQ ID NO:51, SEQ ID NO:53, SEQ ID NO:55, SEQ ID NO:55, SEQ ID NO:60, SEQ ID NO:62, SEQ ID NO:64 or SEQ ID NO:66.

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- 7. A DNA vector containing the nucleotide sequence of Claim 1, 2, 3, 4, 5, or 6.
- 10 8. An expression vector containing the nucleotide sequence of Claim 1, 2, 3, 4, 5, or 6 operatively associated with a regulatory nucleotide sequence containing transcriptional and translational regulatory information that controls expression of the nucleotide sequence in a host cell.

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- 9. A genetically engineered host cell containing the nucleotide sequence of Claim 1, 2, 3, 4, 5, or 6.
- 10. A genetically engineered host cell containing the
 20 nucleotide sequence of Claim 1, 2, 3, 4, 5, or 6 operatively
 associated with a regulatory nucleotide sequence containing
 transcriptional and translational regulatory information that
 controls expression of the nucleotide sequence in a host
 cell.

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- 11. An isolated SCARECROW protein.
- 12. The protein of Claim 11 having the amino acid sequence shown in FIG. 5E (SEQ ID NO:2).

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- 13. A SCARECROW protein lacking one to four of the following motifs delineated in PIGS. 13A-F: MOTIF I, MOTIF II, MOTIF III, MOTIF VI, Or MOTIF VI.
- 35 14. A p lyp ptid corr sp nding to MOTIF I, MOTIF II, MOTIF IV, MOTIF V or MOTIF VI of the SCARECROW prot in as d lineat d in FIGS. 13A-F.

15. An antibody that immunospecifically binds the prot in or polypeptid of Claim 11, 12, 13 or 14.

- 16. An anti-idiotypic antibody that mimics an epitope of the 5 SCARECROW protein.
 - 17. A plant engineered to overexpress or underexpress the SCARECROW protein, so that cell division is modified and root development is altered

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- 18. A plant engineered to overexpress the SCARECROW protein, so that cell division is increased in roots, resulting in thicker root development.
- 15 19. A transgenic plant containing a transgene having the nucleotide sequence of Claim 1, 2, 3, 4, 5, or 6.
 - 20. A transgenic plant containing a transgene having the nucleotide sequence of Claim 1, 2, 3, 4, 5, or 6 operatively
- 20 associated with a regulatory nucleotide sequence containing transcriptional and translational regulatory information that controls expression of the nucleotide sequence in a transgenic plant cell.

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- 25 21. The transgenic plant of Claim 19, in which the transgen encodes an antisense molecule that suppresses expression of endogenous SCARECROW gene product, so that cell division is decreased in roots, resulting in thinner root development.
- 30 22. A genetically engineered plant in which the endogenous SCARECROW gene is disrupted or inactivated so that cell division is decreased in roots, resulting in thinner root development.
- 35 23. A transgenic plant containing a transgen encoding a g n f int rest op ratively associat d with a SCARECROW promoter, so that the gene of interest is express d in roots.

24. The transgenic plant f Claim 23, in which the gene of inter st encod s a gene product that confers h rbicide, salt, pathogen, or ins ct r sistanc.

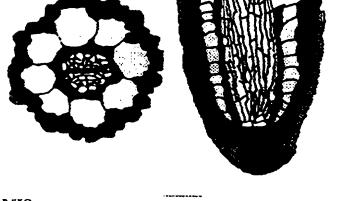
- 5 25. A transgenic plant containing a transgene encoding a gene of interest operatively associated with a SCARECROW promoter, so that the gene of interest is expressed in stems.
- 26. The transgenic plant of Claim 25, in which the gene of 10 interest encodes a gene product that increases starch, lignin or cellulose biosynthesis.
- 27. A plant engineered to overexpress or underexpress the SCARECROW protein so that the stem or hypocotyl gravitropism 15 is altered.
 - 28. The plant of Claim 28, which is less susceptible to lodging than a wild-type plant.

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EPIDERMIS

CORTEX

CORTEX/ENDODERMAL INITIAL

ENDODERMIS

EPIDERMAL/ROOT CAP INITIAL

PERICYCLE

QUIESCENT CENTER

ROOT CAP

FIG.1A

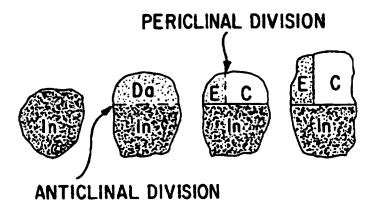


FIG.1B

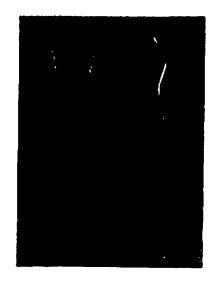


FIG.2A



FIG.28

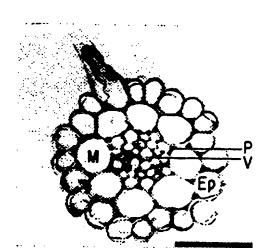


FIG.2C

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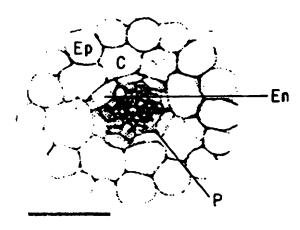


FIG.2D

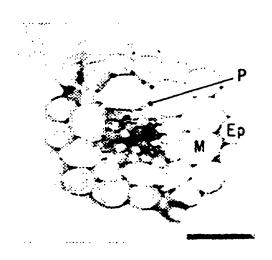


FIG.2E

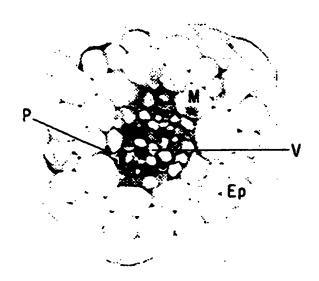


FIG.2F



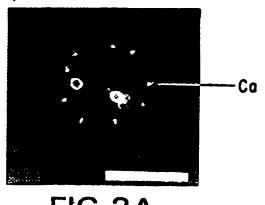


FIG.3A

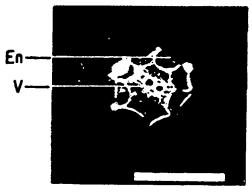


FIG.3D

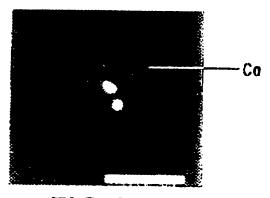


FIG.3B

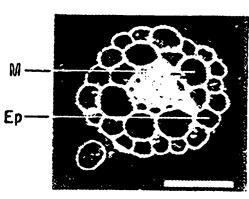


FIG.3E

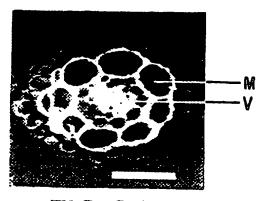


FIG.3C

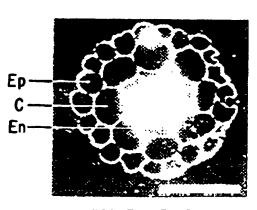


FIG.3F

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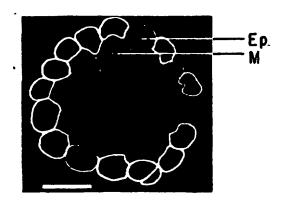


FIG.4A

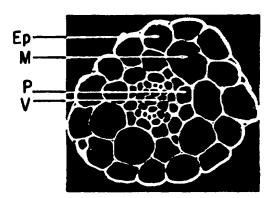


FIG.4D

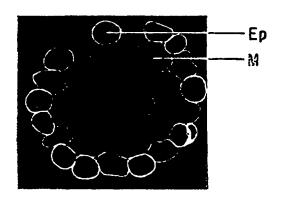


FIG.4B

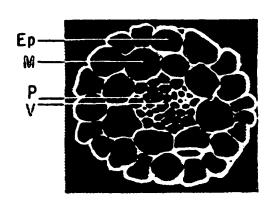


FIG.4E

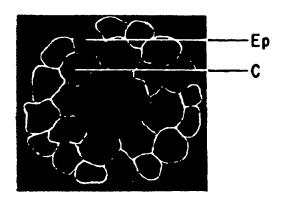


FIG.4C

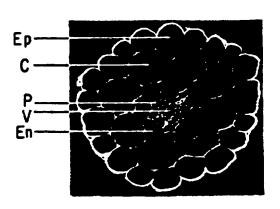


FIG.4F

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24 64 104 144 184 224 264 304 TACTGTATGGGTTGAC 120 TICCTCTCCTCTCACCTTCTCCAAACTTTCGAACCTCTTATCAGATCTCCAACAATCCTTCTCCTCCACAACAGCAACAGCACCACCAA \underline{S} \underline{S} \underline{S} \underline{S} \underline{S} \underline{D} \underline{P} \underline{Q} \underline 01 SI SI ⋖ 2 SI 91 /ACCGCGGAGGCTITAAGAGGAGGAAGGAGGATTAAGAGGCAGAAGCAAGAGGAAGAAGGATTACACCTTCTCACATTGCTGCTACAGTGTGCTGAAGCT ITCTACCGATGCACCACCACAGACAGTGACGCCCACTGT z ITCAGACCCTGCTGCTCGGAATGTTATGATGTCCGTACAATGCATCAACACTCTTCATCTTCTTCTTCTGCTTCACCT \underline{S} D R G G R N V M M \underline{S} V \underline{Q} P M D \underline{Q} D \underline{S} \underline{S} \underline{S} \underline{S} \underline{S} \underline{S} A \underline{S} P IATCAGAGACCTTATCCATCCTCAACTTCAGTCTCAACTTATCCAAAACGTTAGAGACATTATCTTCCCTTGTAACCCAAATCTCGGTGCT œ \equiv 8 ا ہ 0 S 21 م ا ξŦ =8 2 ~ 1 SI පු പ SI TCCTCAACCT ≥ 8 w SI AGTAGCAGCAGCAACAACCGTGCTCCTCCTCCTCCTTTAGTGATGGTGAGAAAAAATTAGCT ပ Z 2 CAACAACAACAACATAAGCCTCCTCCTCCTCCGATTCAGCAGGAAAGAGAAAATTCT \underline{Q} \underline{Q} ပ 8 SI \$ 8 TGATCCTI 0 P § 5 rcccstgtctctcaccttct r r v <u>s</u> h l l ⊑╙ 70000T 2 CTCCCATCTCTCATGCTCCT SI ဗ္ဗ SI SUBSTITUTE SHEET (RULE 26)

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TITACAGOGAATCAGGCGATTCAAGAAGCATTTGAGAAGACAGTGTGCACATCATTGACTTGGACATCATGCAGCGATTCAATGGCCTGGTTTATTCCACATTCTTGCTTCTAGA F T A N O A 1 O E A F E K E D S V H I 1 D L D 1 M O G L O W P G L F H I L A S R

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TCGTGTCTCGCAATTTACCCCCCTTCCCCTTCACCCTCCACCCTCAAACCCATACCTTGAAAATGGTCTCTGCGTTTCACGTTTTAATGCCATAAGCCCTTTAGTGAAATTCTCACAC

OCTOGAGGACCTOCACACCTGCCACTCCTTCCATCCATCCATCCACCTACACCCAAACCTCTTTCCCATTTCACATAAGCTTCCCCTGCCTTTTGAGTTCTGC

504

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546

586

626

653

FIG.5A-2

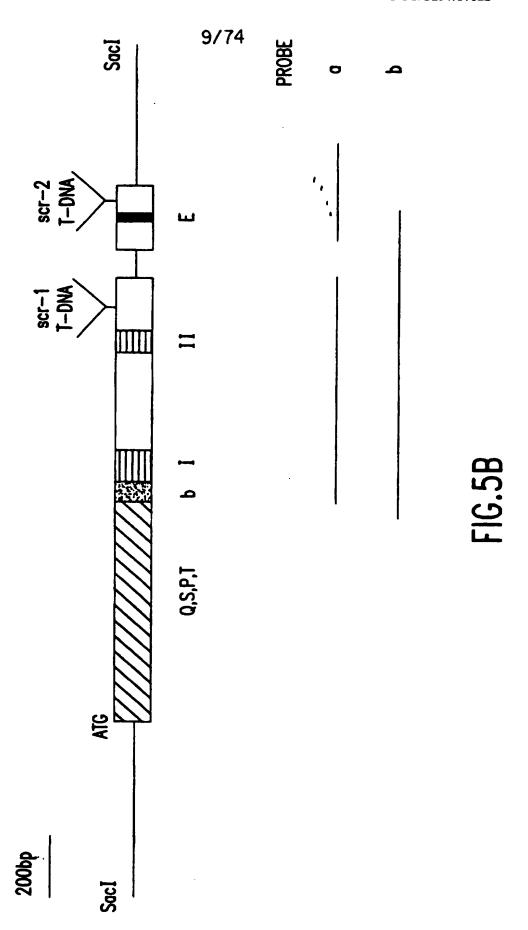
TICGGAATGAACAGATTAGCTCCTAAAGTTGTGACAGTAGTGGAGAATTTGAGCCACGCTGGTTCTTTAGGAACATTTGTAGAGCAATACATTACTACTCTGCACTCTTTGA

CICACIGGGAGCAAGCTACGGCGAAGAGAGGAGAGAGAGACAIGICGICCAACAGCAGCTAITAICGAAAGAGAIACGGAAIGIATIAGCGGIIGGAGGACCAICCAGAAGCGGIGAAGI ~ S م ပ ပ > × > z <u>~</u> <u>—</u> **×** S 1 1 0 0 ш > > = œ u u S u u ပ ≻ S ≪ ပ GAAGITICACAGCICCACCACAAAAIGCCAACAAIGIGGGIIIAAAGGIAIAICIIIAGCIGGAAAIGCIACAGCACAAGGACAGGIGGGAAAAIGIIICCTICGGAIGGIIACAC K F E S W R E K M Q Q C G F K G I S L A G N A A I Q A I L L G H F P S D G Y I

TITGGIIGAIGAIGAIGCIACACTIAGCIIGGAIGGAAGAICIIICGIIACICACIGCIICAGCIIGGAGGCCICGIICIIAGIIIICIICIICIITIICACAAACAAIGIGCCCAIA L v D d n g I l k l g w k d l s l l i a s a w i p r s stop

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			1
			1
SCR bZIP-	like domain	PAVQTNTAEALRERKEE I KRQKQ	D
		11 1 1 1 1 1 1 1 1 1 1 1 1 1 1 1 1 1 1 1	
GCN4	(yeast)	LKRARNTEAARRSRARKLQRMKQ	L
TGA1	(Arabidopsis)	RRLAQNREAARKSRLRKKAYVQQ	L
C-Fos	(mouse)	IRRERNKMAAAKCRNRRRELTDT	L
c-JUN	(human)	RKRMRNRIAASKCRKRKLERIAR	L
CREB	(human)	VRLMKNREAARECRRKKKEYVKC	L
Opaque-2	(maize)	KRKESNRESARRSRYRKAAHLKE	L
OBF2	(maize)	MRQIRNRDSAMKSRERKKSYIKD	L
RAF - 1	(rice)	RRMVSNRESARRSRKKKOAHLAD	1

FIG.5C

SCR VHIID domain	domain
SCR	1 AFEKEDSVHIIDLDIMQGLQWPGLFHILASRPGGPPHVRLTGL
F13896	AVKNESFVHIIDFQISQGGQWVSLIRALGARPGGPPNVRITGI
237192	AMEGEKMVHVIDLDASEPAQWLALLQAFNSRPEGPPHLRITGV
225645	AIKGEEEVHIIDFDINQGNQYMTLIRSIA
D41474	IHVIDFXLGVGGQWASFLQELAHRRG

F16.50

VHIIXFXLMQGLQWPALMDVFSARKGGPPKLRITGI

T18310

Thr Protyr Gly Thr Ser Ala Gln Arg Val Ala Ala Tyr Phe Ser Glu Ala Met Ser Ala

F1G. 5E-1

ValTrpValAspAlaIleIleArgAspLeuIleHisSerSerThrSerValSerIlePro ProglnThrPheGluProLeuTyrGlnIleSerAsnAsnProSerProProGlnGlnGln GlnGlnHisGlnGlnGlnGlnGlnHisLysProProProProIleGlnGlnGln ProllavalginThrAsnThrAlaGluAlaLeuArgGluArgLysGluGluIleLysArg GlnLysGlnAspGluGluGlyLeuHisLeuLeuThrLeuLeuLeuGlnCysAlaGluAla ThrserserglyserserserAsnAsnArgGlyProProProProProProProPro LeuValHetValArgLysArgLeuAlaSerGluMetSerSerAsnProAspTyrAsnAsn **ProglnGlnProProSerLeuThrAlaAlaAlaThrValSerSerGlnProAsnProPro** LeuSerValCysGlyPheSerGlyLeuProValPheProSerAspArgGlyGlyArgAsn GlnLeuIleGlnAsnValArgAspIleIlePheProCysAsnProAsnLeuGlyAlaLeu **MetAlaGluSerGlyAspPheAsnGlyGlyGlnProProProHisSerProLeuArgThr SerSerArgProProArgArgValSerHisLeuLeuAspSerAsnTyrAsnThrValThr ValMetMetSerValGlnProMetAspGlnAspSerSerSerSerAlaSerProThr** LeuGluTyrArgLeuArgSerLeuMetLeuLeuAspProSerSerSerSerAspProSer ValSerAlaAspAsnLeuGluGluAlaAsnLysLeuLeuLeuGluIleSerGlnLeuSer **GluArgGluAsnSerSerThrAspAlaProProGlnProGluThrValThrAlaThrVal**

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F16.5E-2

SerArgSerGlyGluValLysPheGluSerTrpArgGluLysMetGlnGlnCysGlyPhe LysGlylleSerLeuAlaGlyAsnAlaAlaThrGlnAlaThrLeuLeuLeuGlyMetPhe **ProSerAspGlyTyrThrLeuValAspAspAsnGlyThrLeuLysLeuGlyTrpLysAsp** TyrSerAlaLeuPheAspSerLeuGlyAlaSerTyrGlyGluGluSerGluGluArgHis ValValGluGlnGlnLeuLeuSerLysGluIleArgAsnValLeuAlaValGlyGlyPro SerAspAlaHisThrLeuTrpLeuLeuGlnArgLeuAlaProLysValValThrValVal GluGlnAspLeuSerHísAlaGlySerPheLeuGlyArgPheValGluAlaIleHisTyr **LeuSerLeuLeuThrAlaSerAlaTrpThrProArgSerSTOP**

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ArgLysArgGluAlaValAlaValHisTrpLeuGlnHisSerLeuTyrAspValThrGly

GluAlaLeuGlnAlaThrGlyLysArgLeuSerAspPheThrAspLysLeuGlyLeuPro

PheGluPheCysProLeuAlaGluLysValGlyAsnLeuAspThrGluArgLeuAsnVal

LeuAlaSerArgProGlyGlyProProHisValArgLeuThrGlyLeuGlyThrSerMet

ValHisIleIleAspLeuAspIleMetGlnGlyLeuGlnTrpProGlyLeuPheHisIle

LysPheSerHisPheThrAlaAsnGlnAlaIleGlnGluAlaPheGluLysGluAspSer

ArgLeuLeuAsnSerCysLeuGlyIleTyrAlaAlaLeuProSerArgTrpMetProGln

ThrHisSerLeuLysMetValSerAlaPheGlnValPheAsnGlyIleSerProLeuVal

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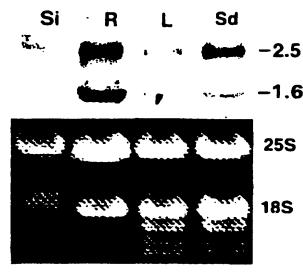


FIG.6A

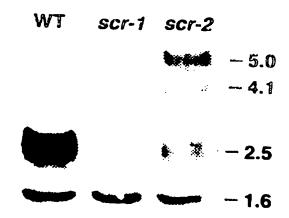


FIG.6B

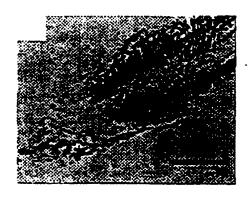


FIG.7A



FIG.7C

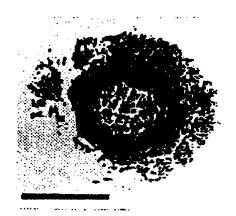


FIG.7B

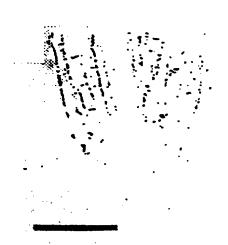


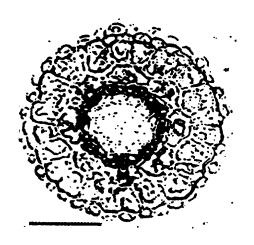
FIG.7D



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FIG.7E





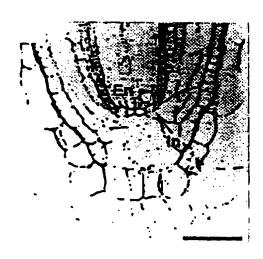


FIG.7G

	50	40	30		20		10	
	1234567890	234567890	7890	123456	567890	1234	67890	12345
50	ATATCTTGTA	CTTATATGC Y M H	TCTT	TGAGCT E L	GGGTCC	CAAC	GAGCC	GGCAO G T
100	AATGGAGCTA N G A I	GAATCTGCT S A	GTTA Y	AATTCG F G	ATTICA F K	CCTTA P Y	CCTGC C	TGAAG E A
150	CGATTTCCAG D F Q	CACATTAT H []	TTTG F V	GAAAGT E S	NAGAAC N	TGTGA V K	GAAGC E A	TAGCT(
200	TTGGTGCTAG G A R	CCGTGCTC R A L	TTTG L	GGTGAG V S	CAATG Q W	GTGGT G	TCAAG Q G	ATTTC'
250	GATCCGAGAT D P R S		TAAC T	TTAGGA R I	GAACG N V	CCTCC P P	GTGGA G	ACCTG(
300	AAGACTTGGG R L G		GAGT E L	GGACTT(G L {	AAGGA G	TCGTC R Q	TTGC	CATCGT S F
350	GAGCTGCTTT A A L	_	GTTT (F	TGTTCCC V P	TGCGG C G	AAATG M	GCTG A E	AAGCTA K L
400	AATGGAGAAG N G E A	CAGTTAGA . V R .	IGCT /	TCGAGAA E K	CGAAA E I	GAAGT(E V	CACG (ATGCTG C C
450	TGATGAGAGT D E S	CACATGCC T	TTC A	CTTGTTC - V L	TCCCG (TAACT: N F	CGGT	CCCTCC L A
500	TCAAACACTT K H L	GAGATTGG 1 R L V	TTG T	AGATAGA D R	CACAG /	AGAAT(N H	GTGG / V E	GTAACT V T
550	ACAAACACTG N T A		GCA A Q	rggttga V E	SACTC 1	GTTGT0 V V	AAAC (N \	GTCACC S P
600	CTTGGCAGIT	ACCATTA (CAA T	TCGAGA	GTTT G	CCCCG	TTCT 1	CCCCT

FIG.8A

10	20	30	40 50	
1234567890	1234567890	1234567890	1234567890 1234567890	
TTCGAATCAA F E S I	TAGATGTGAA	ACTCGCTAGA L A R	GATCACAAGG AAAGGATCAA D H K E R I N	650
	CATTGTTTGG H C L A		TGTGAATCTT ATAGCTTGTG V N L I A C E	700
	AAGAGAAGAG R E E		CACTAGGGAA ATGGAGGTCT L G K W R S	750
CGGTTTCACA R F H M			CCTTTGAGCT CGTATGTGAA P L S S Y V N	800
CGCAACAATC A T I		TTGAGAGTTA E S Y	TTCAGAGAAG TATACACTTC S E K Y T L E	850
AAGAAAGAGA E R D	TGGAGCATTG G A L	TATTTAGGAT Y L G W	GGAAGAATCA ACCTCTTATC K N Q P L I	900
ACTTCTTGTG T S C A		ACTAATAAAA	ACCTTGTTCG GTTTCAGAAG	950
AGATTAGAAA	CTTCTTTTAA	AGTTTGCAGA	ATCTGTTTGT AAAAGTAAAA	1000
CTCATGCATG	ATCCGNAGGA	ACAAGTTGTC	AAATGTTGTA GTAGTAAGTG	1050
ATATGTTGAT	GACCCAAAAA	ΑΑΑΑΑΑΑΑ	AAAA	1085

FIG.8B

			0 40 50	
1234567890	1234567890	1234567890	1234567890 1234567890	
GCTATGGAAG	GAGAGAAGA	T GGTTCATGTC	ATTGATCTCG ATGCTTCTGA	50
			IDLDASE	
GCCAGCTCAA	TGGCTTGCT	T TGCTTCAAGO	TITTAACTCT AGGCCTGAAG	100
P A Q	WLAI	LQA	FNSRPEG	
GTCCACCTCA	TTTGAGAAT	CACTGGTGTTC	ATCACCAGAA GGAAGTGCTT	150
PPH	LRI	TGVH	HQKEVL	
			•	
GAACAAATGG	CTCATAGAC	CATTGAGGAA	GCAGAGAAAC TCGATATCCC	200
E Q M A	HRL	I E E	AEKLDIP	200
GTTTCAGTTT	AATCCCGTTC	TGAGTAGGTT	AGACTGTTTA AATGTAGAAC	250
		SRL		200
·				
ACTTGCGGGT	TAAAACAGGA	GAGGCCTTAG	CCGTTAGCTC GGTTCTTCAA	300
1 R V	K I C	FAIA	V S S V L Q	300
,	K 1 0		, , , , , , , , , , , , , , , , , , ,	
TTGCATACCT	TOTTOGOCTO	TCATCATCAT	CTCATGAGAA AGAACTGCGC	350
INTE	1 A C	D D D		220
	L A 3	ט ט ט	LMRKNCA	
TTTACCCTTT		CTACTCCACT	TGACTTGCAG AGAGTTCTAA	400
L R F	O N N O	CIACIGGAGI	IGACTIGCAG AGAGTICTAA	400
LKF	UNNP	2 G A	DLQRVLM	
TOATOACOCA	TOOOTOTOOA	0070100010		
TOATGAGCCA	IGGCTCTGCA	GCTGAGGCAC	GTGAGAATGA TATGAGTAAC	450
M 2 H	G S A	ALAR	ENDMSN	
	.=			
AACAA IGGGI	ATAGCCCTAG	CCCTCACTCC	GCCTCATCTT IGCCTTTACC	500
NNGY	SPS	GDS	ASSLPLP	
AAGTTCAGGA	AGGACTGATA	GCTTCCTCAA	TGCTATTTGG GGTTTGTCTC	550
SSG	RTDS	FLN	A I W G L S P	
			- '	
CAAAGGTCAT	GGTGGTCACT	GAGCAAGACT	CAGACCACAA CGGCTCCACA	600
KVM	v v t	E Q D S	DHNGST	

FIG 9A

	50	40	30	20	10
				1234567890	
650			ATCACTTTAC S L Y	GGCTATTAGA L L E	CTAATGGAGA L M E R
700	_	TCAAGATAGG	CAAGAACGTC	ACAAAAGTTC T K V P	
750	CTGCGAGGGA C E G			CTTCGGGGAG F G E ,	
800				GAGAAAGACA E R H	
850				GGTTTTGGGA G F G N	
900	TAGAATCAAG R I K			ATTGCTTCAA L L Q	
950	CTCTATACTC L Y S				GAAGAGAGCG E E S G
1000	AGTTTGTCTT	GATATATTAC		TCGAGATCCA W R C R	GGTATCAGCT V S A
1050	CATGGGGACA	TTTTTGTATA	GTCCCTTTCT	TATGAGCAGA	CTATTTTGGT
1100	ATGCTATTTT	CTGTCTCTTT	TGGTGACTTT	TGTTTTGTGA	CAATCTTAGT
1150	TGTGTTGGTT	AAAGCCTTTG	CTCTGCATGT	CTTCTACTGC	GCCTTAAATG
1200	ATTTGAGCTG	ACCAAATCCA	GTAATACCAA	TGGTGTGGGT	CAATTTGGTC
1231		r.	CGGCTCGTGC	ATTIGATGAT	AAGATAACTA

FIG.9B

				AAAAAA	CTCACTTAAA
3	TGTTAAGGTT	TGGTTAAATT	TCAGTTCAAA	TGTTCATATT	AGTCTTTACA
1300	AGTGTGAATG	CTATTTGCTA	GGTTCTAAAC	TTAGAGTTTT	ATAAAAATAA
1250	TCCCTATTAT	TTCTTTTTT	GAATTTTTCT	ACTTATGGAA	CTAATCTAAC
1200	GTACTAAAAG	GTGATCACAG	GAGGTTAGGG	ATTATTACCA	ATCTCCATGT
1150	TGGTTAAATC	ATTGTGTAAC	ATCTTGGGTT	TTTTACTGTA	ATAATTATGT
1100	TTCATAAAAC	GTCTTTATGT	AGAGCATATA	ATAAGTGACA	TGGAGGTAAG
1050	TGCTTCAGCT	GCTTAATCGT	GAGGAGAAAA	TTTTTGCTGG	GTGAGCTCCA
1000	GAAGAAATGG	CAAGCTTAAA	GCAATAAGTA	CAACAATATT	CCTGATAAAG
950	ATATACAAAA	GTAACCAACA	GAGTGCTAAA	CAAAACCAAT	GGATTCAATC
006	GATGATGGCT	GAGCAAGGAT	GGAAAATGGA	CGAGGCTGCG	TAGAGAGATA
850	GAAGAACGGA	TTGCGAAGGA	ACATTGTTGC	GACATAGTCA	TCTCGCTAGA
800	AAAGACAGTG	ATGAATGTAG	CCAAGAGAGG	CAAGAGAAAG	ATGACACTTC
750	GTCTCTAGAC	CAGTTTTCGA	TACTACTCAG	GGCTTACGAA	GATTCATAGA
700	TTCTTTCCCA	CACTTCACCG	TGAACACAAA	GAACAAGACG	CACGGTCGTT
650	ACCCGCTTGT	AAAAGCTTAA	TCACATGGTC	ACGAGCTACT	AACCAGCGGG
009	CACAACAGTA	ACGAGAGTGT	CACATGCCTG	CCAACTTCAC	ACTTTGCATT
7 0	TAATCAGTGA	GGAGAAACCT	TTGCAAACCA	CAACACTCGG	GTCTCTCCAT
500	GACTTCGATT	TGCCTTCAAA	TTCAAAGCAA	ATCCTTCAAA	ATAATGGAGT
450	CTCGCAGAGG	ACTCGAGCAA	TCAATCTAAG	CTAAGAATCA	CATTGGAGGG
4 0 0	TCCAACGCTC	CCTGAATCAG	AATTGATGAC	GGTTAACAGG	CCTCGCCTGA
250	TGGTAAACGA	CTGAGTTGCC	CGAAGCATTG	GACACTGATA	ACCAATACAT
000	AACCAAGGGA	TTTCGATATA	ACATAATCGA	GAAGAAGTTC	CAAAGGTGAA
200	TTGAAGCAAT	GGTGCGATAC	AGCAGCTAAT	TCGGGTTTTT	TGTTTCAAGT
	AGTCTGCCCT	TCCTGTTTGA	GCTATGCAAG	GAGGCTTGCA	CTTCGGATGA
150	AAAGAGCCTC	ATTGAAATGC	TCTACAGAGC	GGAAAATTCA	GCCCCCTTCA
	CTGCAAGAAT	GAAGGTCTAG	TTACATGGTG	GAATCGCAGC	CCTTCTCAGA
R.	CCAAGGAGAC	TAGTTTCTAT	CTGAGGCAGA	GGTAAATGAG	CITIGICAAT

Zm-sc11

10 20 30 40 50

CCAGGAGGCGTTCGAGCGGGAGGAGCGTGTGCACATCATCGACCTCGACA
Q E A F E R E E R V H I I D L D I

60 70 80 90 100

TCATGCAGGGGCTGCAGTGGCCGGCCTCTTCCACATCCTTGCCTCCCGC
M Q G L Q W P G L F H I L A S R

FIG.11A

10	20	30	40 50	
			1234567890 1234567890	
CACGCGTCCG	TCAAAGGATA	CAACCATGTA	CACATAATTG ACTITICCCT	50
HASV	KGY	NHV	HIIDFSL	
GATGCAAGGT	CTCCAGTGGC	CGGCACTCAT	GGATGTCTTC TCCGCCCGTG	100
	LOWP			
•		7, C N	o v , o x k c	
ACCCTCCCCC	ACCAAACCTC	CCAATCACAC	GCATTGGCCC GAACCCAATA	150
G G P			I G P N P I	130
0 0 1	FNL	K I I G	IGPNPI	
CCTCCCCCTC	ACCACCTCCA	TOARCTOOCA	ATTOCCOTCC CCAACTATCC	200
			ATTCGCCTCG CCAAGTATGC	200
GGKD	ELH	E V G	IRLAKYA	
1010700070	0071700107	7010777001		
			GGGAGTCTGT GTCGATCAAC	250
H S V	GIDF	TFQ	GVCVDQL	
TTGATAGGTT			AACCAATCAA AGGAGAGGCA	300
DRL	C D W	MLLK	PIKGEA	
GTTGCCATAA	ACTCCATCCT	ACAACTCCAT	CGCCTCCTCG TTGACCCAGA	350
VAIN	SIL	QLH	RLLVDPD	
TGCAAACCCA	GTGGTGCCCG	CACCAATAGA	TATCCTCCTC AAATTGGTCA	400
			ILLKLVI	100
	• • • • •		1	
TCAAGATAAA	CCCCATGATC	TTCACCCTCC	TTGAGCATGA GGCAGATCAC	450
KIN	P M I	F T V V	E H E A D H	430
	1 191 1		C II C A D II	
AACACACCAC	CACTACTACA	CACCTTCACT	AATGCCCTCT TCCACTATGC	500
				500
NKPP	L·L E	וזא	NALFHYA	
0100170777	01070777			
			TCGTTGTACC AGTGGTAGAG	550
TMF	DSLE	AMH	RCTSGRD	
101701000				
			TTCGAGGTGA GATTTTTGAC	600
I T D	SLT	EVYL	RGEIFD	

FIG 11B1

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10	20	30	40 50	}
1234567890	1234567890	1234567890 123	34567890 1234567890	
		TGCACGCACC GA A R T E	ACGTCATG AGTTGTTTGG R H E L F G	650
		CCTATGCTGG GCT	TAACTCAA GTGTGGTTCG T Q V W F D	700
	GGTTGACACG V D T		TTGATCCA TGTGACATCC	750
		CATCCTAGTG TGT	IGATGGCA GCCTTGCACT D G S L A L	800
AGCGTGGCAT A W H		TATATGTGCC AAC	CAGCTIGG TGTGTGACAG A W C V T G	850
	TGCCAGTTCC A S S		ATCTGTAA GGGTACAAAT L C K G T N	900
		CCGTAATGGA CCC R N G P	CATGGAGT AGCAGGAAGA M E X	950
ATAACCATGT	CATGAGCAAA	TCGATCAAGT AAT	TAAAATGC ACTGATGACA	1000
TGCATGGTGA	TCTAAAGTTT	TTTTGCGTGA ATO	STGCAATG ACGAATTGTT	1050
CAATTTGAAT	AACCTAATCA	TGAGACTCAA AAA	MAAAAAA AAA	1093

FIG.11B2

CAAGGAGGCC	GCGTCACCTC	TTCTCTGACC	GCGCTTCTTG	TGACTTTGAT	TTGCCCACCG	ACGCCTTTCA	CCAGGATAAC	TCAATGCCGT	TCTGGTGATG	TGCACCACCG	AGGTTGTCGT	
GAAGCCCTTC CTCCGCTCCG CCTCCTACCT CAAGGAGGCC	GGCTCCTCCG		CCGCAACAAG	TCCATGTCAT	TTGCAGGAGC	GITGAAGCTC	TGCACCTTAC	CCTTTCGAAT	TATTTCTTCC	GCTCTGCTCG	CTTTGTCCTA	
CICCGCICCG	CAGCCACCAT	AGCTTGCAGC	ACTAACTTTA	ACTUCCTGCA	GGCTTCCTTC	CCTTGCCGTT	CCACTGGAGC	GCTCAGAATT	CTGCGGAATC	CCTGTTGGCT	GGTGAAACAG	
GAAGCCCTTC	CACTCGCCGA	GTTGCCCTCA	GCTCCAGTTC	TGGCATGGCA	GTGGTCAGTG	GGAGGTATGG	TTCTCACCAT	TTGCCGCAGA	GCATTCAATC	TGTTAGCCTC	TTCTTCGGTT	
CCCAACTIGG	CICCICCICG	GCCGCTCGAC	TGTCACCTGT	Atgagattgg	CICGGIGITG	CCGGGGAGCT	TGTCGACTGC	CTCTCTCAGT	CAGTCTTGAT	AAGTTGTTGC	CTGCCAGCGA	GGCTATTGAT

F16.12A

50	100	150	200	250	300	350	400	450	200	
CAGCAGTATA	TACCCTCTAG	CTAATTGAGC	AGACTGACAC	CGARARGCAR	AACATTTAAT	CAAACGGTGG	GCCAAGATGA	GCAGCTCCGC	GAGGCAGTCT	
TACAGAGCAA CAGCAGTATA	TAGGTTAAAT	ATGATCATGG	TTAGCTATAG	TAAGCTGCAA	TTTCATTTGC	TCGATGCTTG	GATCAGCACC	GAGCGTAAGA	CCCGCTTCAG	
TTTTTTTT	AACCATTTGA	TTCCAATGAG	AGCAACATCA	GGCTAGCTAA	AAGACAACAA	ACTGGAGTGG	GCTTATGGCT	GCTGCCAGTA	CGGACCTGCA	
TTTTTTTT	TGTACCACAC	TAAGCAGTGT	AACAACCTAA	AAATCCACTA	TCAACAGCTC	AATGGACATT	GTGGAGTGAA	AGCTCCCCAC	ATGGAATCCT	
TTTTTTTT	ATATTAATTC	TCTCTACTCA	AGAGCATGGC	CAATATTCCT	TATGAAGAGT	TGCAAGAATA	TGGAACCTTG	TATGGATACA	GTTTCTCCAC	၁၅

F16.12B

MAESGDFNGGQPPHSPLRTTSSGSSSSNNRGPPPPPPPPPLVMVRKRLASEMSS MKRDHHQFQGRLSNHGTSSSSSISKDKMMMVKKEEDGGGNMDDELLAV MKRDHHHHHQDKKTMMMNEEDDGNGM-DELLAV	 NPDYNNSSRPPRRVSHLLDSNYNTVTPQQPPSLTAAATVSSQPNPPLSVCGFSG -LGYKVRSSEMAEVALKLEQLETMMSNAQEDGLSHLATDAAHYNPSELYS -LGYKVRSSEMADVAQKLEQLEVMMSNVQEDDLSQLATETVHYNPAELYT	LPVFPSDRGGRNVMMSVQPMDQDSSSSASPTVWVDAIIRDLIHSSTSVSIPQL 	IQNVRDIIFPCNPNLGALLEYRLRSLMLLDPSSSSDPSPQTFEPLYQISNNPSP PSPEICGFPXSDYDLKVIPXNAIYQFPAIDSSSSSNNQKAI-P
SCR TF1 TF4	SUBSTITUTE SHE	ET (RULE 26)	SCR TF1 TF4

F16. 13A

PQQQQQQHQQQQQHKPPPPIQQQERENSSTDAPPQPETVTATVPAVQTNTAEA

-NKRLKSCSSPDSMVTSTSTGTQIGGVIGTTVTTTTTTAAAES

SCR TF1 TF4

-GGGGDTYTTNKRLKCSNGVVETTTATAES

LRERKEEIKRQKQDEEGLHLLTLLLQCAEAVSADNLEEANKLLLEISQLSTPYG LSMYNELRQIVSIQG LSMYNELRQIVSIQG TRSVILVDSQENGVRLVHALMACAEAIQQNNLTLAEALVKQIGFLAVSQI TRHVVLVDSQENGVRLVHALLACAEAVQKENLTVAEALVKQIGFLAVSQI QLGKPFL TRHVVLVDSQENGVRLVHALLACAEAVQKENLTVAEALVKQIGFLAVSQI	TSAQRVAAYFSEAMSARLLNSCLGIYAALPSRWMPQTHSLKMVSAFQVFNGISP GTSPT-GPELLTYMHILYEACP GTSPT-GPELLTYMHILYEACP DPSQRIAAYMVEGLAARMAASGKFIYRAL-KCKEPPSDERLAAMQVLFEVCP GAMRKVATYFAEALARRIY-RL-SPPQNQIDHCLSDTLQMHFYETCP RSASYLKEALLLALADSHHGSSGVT-SPLDVALKLAAYKSFSDLSP
LRERKEEIKRQKQDEEGLI LRERKEEIKRQKQDEEGLI TRSVILVDSQENGVI	TSAQRVAAYFSEAMSARLI DPSQRIAAYMVEGLAARMP GAMRKVATYFAEALARR GAMRQVATYFAEALARRRSASYLKEALLLALi
O THE HELL (BULE 26)	SCR 4818 1110 TF1 TF4 3989

29/74 HASVKG--YNHVHIIDFSLMQGLQWPALMDVFSAREGGPP----KLR AMEG--EKMVHVIDLDASEPAQWLALLQAFNSRPEGPP----HLR YFKFGYESANGAIAEAVKN--ESFVHIIDFQISQGGQWVSLIRALGARPGGPP----NVR YLKFAHFTANQAILBAFEG--KKRVHVIDFSMNQGLQWPALMQALALREGGPP----TFR **YLKFAHFTANQAILBAF**QG--KKR**VHVIDF**SMS**QGLQWPAL**MQALALR**PGGPP---**-VFR **VLQF**TN**FTANKA**LLDEIGGMATSCI**HVIDF**NLGVGG**QW**ASFLQE**LA**HRRGAGGMALPLLK -RLR CFKFGFLAANGAILEAIKG--EEEVHIIDFDINQGNQYMTLIRSIAELPGKRP--Qeafer - - eervhiidldimoglowfglfhilasr PAG--CRRVHVVDFGIKQGMQWPALLXDLAL

LVKFSHFTANQAIQEAFEK--EDSVHIIDLDIMQGLQWPGLFHILASRPGGPP--

(VHIID)

MOTIF III

F16. 13C

GRNGRTL--WLGEGHIDLWPLQGLLSQGLQRALCARPLGAP---HVF-

Zm-Scl2 Zm-Scl1

Human

SCR 4818 11110 11110 192 371110 193 3935 18310

	30/74
F V	F16.13D
	PK
IV (DIN LSDFTDK LGKLAEM LEQLAED LIEEAEK LAQLAEA LAHLAEA LSQFAAE LSQFAAE LAKYAHS	CLQRLAPK- HRDRLLRL- 2RDELLHM- CNC-ALRFQNNP 7LG
LQATGKR LELVGQR LELVGQR LEQMAHR LHEVGCK LHEVGCK LHEVGCK LHEVGCK LHEVGIR LHEVGIR	FGSDAHTLWI PDESVTTVNG PDESVTTVNG ASDDDLMRI GRXGGIEKN JGRPGAIDKN VDPDANPVV
LTG LGTSMEA ITGIDDPRSSFARQGG LTGIDDPESVQRSIGG ITG VHHQKEV LTGIGPPAPDNSDH LTGIGPPAPDNFDY LTAFMSTASHHPLE ITGIGPNPIGGRDE LPGLHTLS	RKREAAVHWLQHSLYDVTGSDAHTLWLLQRLAPK
SCR 4818 1110 3935 TF1 TF4 3989 18310 Human	SCR 4818 1110 3935 TF1 TF4 18310

F1G. 13E

LLSKEIRNVLAVGGPSRSGEVKFE-SWREKMQQCGFKGIS- CLAREVVNLIACEGVEREERHEPLGKWRSRFHMAGFKPYP-	CLARDIVNIVACEGEERIERYEAAGKWRARMMAGFNPKP-	LFGEEIKNIISCEGFERRERHEKLEKWSQRIDLAGFGNVP-	-LGXQICNLVACEGPDRVERHETLSQWGNRFGSSGLAPAH-	-LGKQICNVVACDGPDRVERHETLSQWRNRFGSAGFAAAH- -LRGEIFDIVCGEGSARTERHELFGHWRERLTYAGLTOVWF		O	LSSYVNATIKGLLES-YS-EKYTL-EERDGALYLGWKNQPLITSCAWR*	MSAKVTNNIQNLIKQQYC-NKYKLKEEM-GELHFCWEEKSLIVASAWR*	LSYYAMLQARRLLQGCGF-DGYRIKEES-GCAVICWQDRPLYSVSAWRCRK*	LGSNAFKQASMLLSVFNSGQGYRV-EESNGCLMLGWHTRPLITTSAWKLSTAAH*	IGSNAFKQASMLLALFNGGEGYRV-EESDGCLMLGWHTRPLIATSAWKLSTN*	ADCLL-KRVQVRGFHV-EKRGAALTLYWQRGELVSISSWRC*	DPDEVDTLKDQLIHVTSLSGSGFNILVCDGSLALAWHNRPLYVATAWCVTGGNAA
SCR 4818	1110	3935	TF1	TF4 18310		SCR	4818	1110	3935	TF1	TF4	3989	18310

10 SSMVGNICKGTNDSRRKENRNGPME*

HIII-Saci CLONE

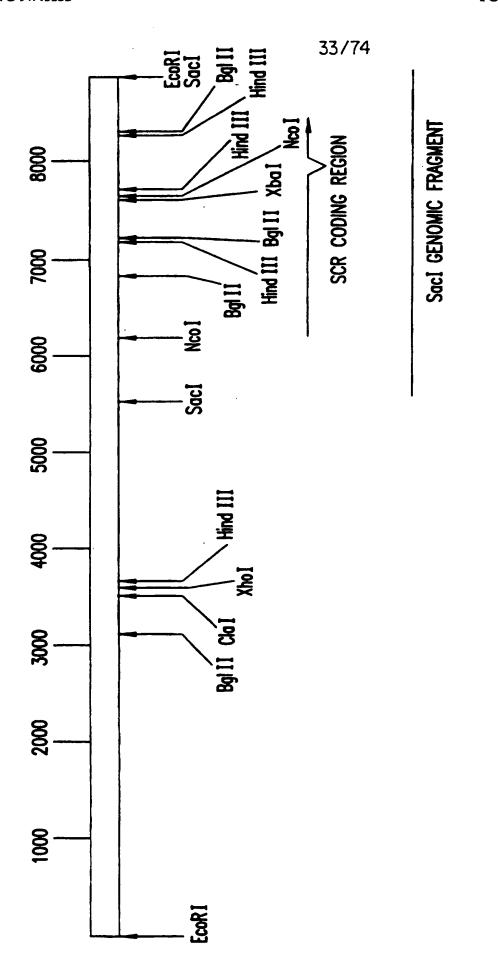


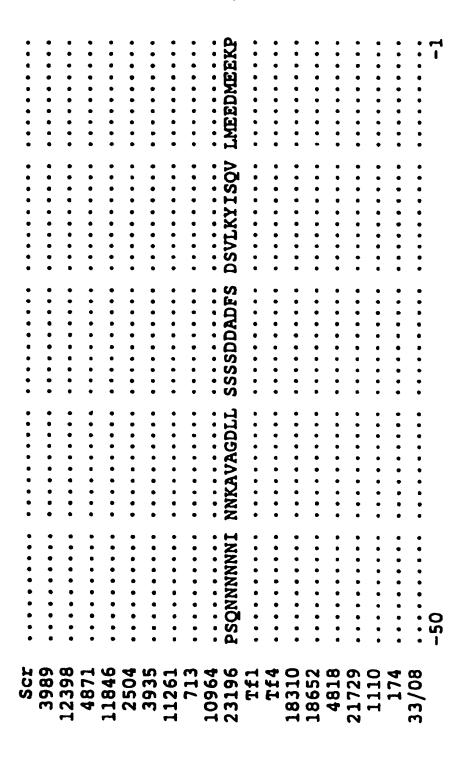
FIG.14

			~	9	5	*	~	m	10	-	<u>-</u>	12	8	~	~	11	*	/	~	7	13	
New	Name	SCR	SRPo3	SRPa6	SRPa5	SRPO	SRP02	SRPa.	SRPa	SRPol	SRPa9	SRPa12	SRPa8	SRPa2	SRPml	SRPal	SRPa	SRPa7	SRPa.	SRPDI	SRPa	
Z	Z	S)	SA	SH	SA	SH	SF	SF	SF	SF	S	S	S	S	S	S	S	Si	S	Si	SF	
		•	•	•	•	•	•	•	•	•	•	S	•	•	•	•	•	•	•	•	•	-
		•	•	•	•	•	•	•	•	•	•	3	•	•	•	•	•	•	•	•	•	101
		•	•	•	•	•	•	•	•	•	•	IIH	•	•	•	•	•	•	•	•	•	1
		•	•	•	•	•	•	•	•	•	•	15K	•	•	•	•	•	•	•	•	•	
		•	•	•	•	•	•	•	•	•	• • • • • • • • •	GFWSWIHMGS	•	:	•	•	:	•	•	•	•	
		•	•	•	•	٠	•	•	•	•		_	•			•						
		•	•	•	•	•	•	•	•	•	•	LLDINDSGFL	• • • • • • • • •	•	•	•	•	•	•	•	•	
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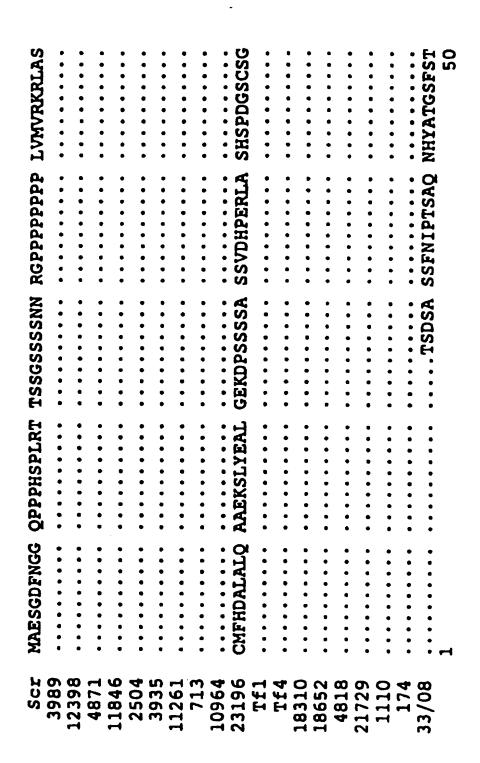
F1G. 15A

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F 16. 15E



F16.15C



F16.15U

danacasuma aartadacca										LHTPMPSNFV FOSTSRSNSV									IAOP	100
I.L.D.SNYNFUT										VDGLENRPSW	GRLSNHGTSS								ATDAEHTDTI	
SSRPPRRVSH					•	•	•	•	•	TTTSSDSHWS	. MKRDHHQFQ	. МККОННИН	•		•	•			TINSATAHWV	
EMSSNPDYNN	•				•	•	•	•	•	GAFSDYASTT	•	•	•	•	•	•	•	•	NSRTTNVATA	~
U	96	39	487	8	2504	93	26	7	960	\mathbf{a}	J.F	•••	31	865	_	72	11	-	33/08	

-16. 15E

U	Ś	VCGFSGLP VFPSDRGGRN	VMMSVQPMDQ	DSSSSSASPT	VWVDAIIRDL
98					
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64					
961	TGGGGGGNSA	VYGSGFGDDL	VSNMFKDDEL	AMOFKKGVEE	ASKELPKSSO
ifl	GNMDDELLAV		AEVALKLEOL	ETMMSNAOED	GI.SHI.ATDAA
£4	NGM. DELLAV	_	ADVAOKLEOL	EVMMSNVOED	DI.SOLATETU
110	•			X .	
52					
4818					
729	•				
10					
74				•	•
	101	•	•	•	150

F16.15F

LDPSSSSDPS		APPPNRLTGK GFPXSDYDLKEYDLK IRMGIGSGIN
LEYRLRSIMI		ETEHHHHSY DPVLPSPEIC COSDXGLFGG
SSTSVSIP QLIQNVRDII FPCNPNLGAL		
QLIQNVRDII		MNSGSKENGS EVFVKTEKKD LDNMLSELNP PPLPASSNGL LDSMLTDLNP P SSNA.
X		LFIDVDSYIP HYNPAELYTW HYNPAELYTW LTSVNDMSLF LTSVNDMSLF
998 999 79	10001 10001 10001	10964 23196 11810 18652 4818 21729 1110

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F 16: 13H

		bzip	bzIP like domain	<u>_</u>		
			otif II (dir	nerization)	\$ 1 1 1 1	
u	POPETUTATU		PAVQTNTAEA LRERKEEIKR	QKQDEEGLHL	LTLLLQCAEA	
86	•	•	•	•	• • • • • • • • •	
O	•	•		•	•	
87	• • • • • • • •	•	AAIFYG	HHHHTPPPAK	RLNPGPVGIT	
8	•	•		•	•	
20	•	•		•	•	
93	•	•	•	•	•	
26	•	•	•	•	•	
71	•	•	•	•	•	
96	•	•	•		•	
19	FP	TAQSNGAKIR	GKKSTSTSHS	NDSKKETADL	RTLLVLCAOA	
If	O	VTTTTTTA	AAESTRSVIL	VDSOENGVRL	VHALMACAEA	
If	•	TTTA	TAESTRHVVL	VDSOENGVRL	VHALLACAEA	
31	•	•	•	•	•	
865	•	•	•	•	•	
481	•	•	•	•		
21729	ITNSNSDWIQ	NLVTPNPNPN	PVLSFSPSSS	SSSSSPSTAS	TTTSVCSROT	
111	•	•	•	•	•	
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	251				300	
		ū	TIC 151			

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	Mot	if II (dime	Motif II (dimerization)		
O	S	KLLLEISQLS	KLLLEISQLS TPYGTSAORV	AAYFSEAMSA	AAYFSEAMSA RLLNSCLGIY
98		•			
39	•		•		
87	EQLVKAAEVI		ESDICLAGGIL ARLNOOLSS	PVGKPLERAA	FYFKEALNNL
84	•				
250		•			
93	•	•	•		
26	•	•	•		
71	•	•	•		
960	•				
19	VSVDDRRTAN	-	SPLGNGSERL	AHYFANSLEA	RLAGIGIOIY
Tf1	IQQNNLTLAE		VSQAGAMRKV	ATYFAEALAR	RIYRLSPPON
If	VOKENLTVAE		VSOIGAMROV	ATYFAEALAR	RIYRLSPSOS
831	•				
65	•		•		
481	•		•	•	L9.
172	VMEIATAIAE	GKTEIATEIL	ARVSQTPNLE	RNSEEKLVDF	MVAALRSRIA
	LSMVNEL	RQIVSIQGDP	SORIANYE	GLAARMAASG	KFIYRALKCK
_	•		•	•	
					350

F16.15J

			← Mot	← Motif III (SCR	VHIID)
u	AALPSRWMPQ	THSLKMVSAF	QVFNGISPLV	QVFNGISPLV KFSHFTANOA	
8	•	•		LYRNKALL	DEIGGMATSC
33	•				
87	Š	_	KSFSEISPVL	_	LLESFHGFHR
84					
250					
•					AMEGEKM
26	•				
_	•				
960	•				
~	ALS.	_	OTYMSVCPFK	KAATTFANHS	MARFTANANT
If	QIDHCLSDT.		MHFYETCPYL	KFAHFTANOA	TLEAFEGKKR
	IDHS	LOI	MHFYETCPYL	KFAHFTANOA	ILEAFOGKKR
_	•		•	HA	SVKGYNH
865	•	•	•	ANVE	ILEAIAGETR
481	PTG	MX	HILYEACPYF	KFGYESANGA	IAEAVKNESF
72	SPVTELYGKE	HLISTQL	LYELSPCF	KLGFEAANLA	ILDAADNNDGGMMI
1110	PPS	AM	QVLFEVCPCF	KFGFLAANGA	ILEAIKGEEE
_	•	•	•	•	•
	351				400

F1G. 15K

Motif IV	VRLTGLGTSM EA	ALDITATE WETSCHOOLS TO			Navglele.	• • • • • • • • • • • • • • • • • • • •		•••••	• • • • • • • •	• • • • • • •					PDNFDYLH.	IGGRDELH	GVDDSOSTYARGGGT.S	GIDDPRSSFAROGELE		AVANNVYGDOT UTDOGGEFOT K			450
1	LASRPGGPPH VRLTGLGTSM	LAHREGAGGM ALDITKTTAE		T.VI. BONDA DI CIVITUENCH	UJCUJATIVICIT JURIN		WOUNDERFORM TOTAL			••••••			がないけいまけば からないが	Addining taken	GGPPV FRLTGIGPPA	GGPPK LRITGIGPNP	GG PPLLRVT	GG PPNVRIT	TIGING TO THE TENT OF THE TENT	LSTRNGKSO SONSPUVKIT		• • • • • • • • • • • • • • • • • • • •	
I (VHIID)	LOWPGLFHI	GGOWASFLOE LAHRA		GOWAST.MOE	K		PAOWT AT TO FNORD			• • • • • • • • • • • • • • • • • • • •	• • • • • • • • • • • • • • • • • • • •	GFOWPALIHRLSLSRP	GI.OWDAT.MOR. T. SUITE		SLUWPALMUA LALRP	SLOWPALMDV FSARE	SSOYMFLIQE LAKRP	SCOWVSLIRA LGARP	SNOYMTLIRS IAELP	GGOYVNLLRT LSTRRNGKSO S			
MOTIT II	IDEDIMO	202		IIDFDIGY			IDLDASE			• • • • • • • • •	• • • • • • • • • • • • • • • • • • • •	IHIIDFGISY	CN	TO TO TO TO	Deme son	IIDFSLMQ	IDFQIAQ	FQISQ	INO	GE			
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ŏ			FLAENVGNUL	TEXTUNA KKKE	AVAVHWE
א		FAAELRIPFE	FNAVSLDAFN	PAESISSSCD	EVVAVSL
ä		• • • • • • • • • • • • • • • • • • • •			
8	FTODNLKHFA	SEINISLDIO	VI. SLDILG	STSWDNCC	TWINTER
v	•				STUANANTO
7					• • • • • • • • • • • • • • • • • • • •
ם סו			• • • • • • • • • • • • • • • • • • • •	NGGAF	APSTWTA
ייני ח	QUARKLIEEA QUARKLIEEA	EXTRIBEGEN	PVVSRLDCLN	VEQLRVK	TGEALAVSSV
2	• • • • • • • • • • • • • • • • • • • •	•	X	KWETITLDEL	MINPGETTUV
7	•				
96					• • • • • • • • •
•			• • • • • • • •	• • • • • • • • •	• • • • • • • •
7	EFRROVIAME	DIVSDIMFRL	STTOLLRNGE	TIOVEDLKLR	OGEYVVVNST.
u	EVGCKLAQLA	EAIHVEFEYR	GFVANSLADL	DASMLET.RPS	DTFAVAVACV
•••	EVGCKLAHLA		GFVANTLADI.	DASMIFTED	TTECHAINCH
31	EVGIRLAKYA		GVCVDOLDRI.	COMM. I VOI	ELECTRONS OF
10	LVGERLATLA		DINE ATMOOR	T TO THE COLA	TOUTUNED
ä	TVCODTOVT			אעמבטדפרנ	FGFAVVNFF
1 (こくられたしていてい		GAALFCI	EVEIEKLGVR	NGEALAVNFP
7	AVGDLLSQLG	DHSISVSFNV	VTSLRLG	DLNRESLGCD	PDETLAVNLA
110	IIGLRLEQLA	EDNGVSFKFK	AMPSKTS	IVSPSTLGCK	PGETLIVNEA
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	Motif	ξ V	· · · · · · · · · · · · · · · · · · ·		
U	··· QHS	•			
96	4	VG			
12398	•	•			
87	AA				
84	•				
20	R	SL			
93	HTFLASD	DDLMRKNCAL	RFHNNPSGVD	LORVLMMSHG	SAAEARENDM
26	HRLOYTP	DE			
71	•	•			
960	•				
13		DE.			
Tf		66			
T£	FELHKLLGRP	GA			
31	LOLHRLLVDP	DA			
865	YVLHHMP	DE			
81	LVLHHMP	DE			
\sim	FKLYRVP	DE.			
11	FOLHHMP	DE			
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	501	•	•	•	550

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	Motif V	1		├─- Motif VI	IN 3:
U	•	LYDVTGSD	AHTLWLLORL	APKVYTVVEQ	APKVVTVVEQ DLSHAGS.FL
98	•	csarappl	PAILRLVKQL	CPKVVVAIDH GGDRADLPFS	GCDRADLPFS
39	•	• • • • • • • • • • • • • • • • • • • •	•	•	•
~	•	SFSHLPLV	LREVKHLSPT	IIVCSDRGCE	RIDLPFSQQL
84	• • • • • • • • • • • • • • • • • • • •		•	0	EADHNKTGFL
50	. NGGAFAPST		DSF	•	•
93	SNNNGXSPSG	DSASSLPLPSSGRT	DSFLNAIWGL	SPKVMVVTEQ	DSDRNGSTLM
26	•	TVSLDSPR	DTVLKLFRDI	NPDLFVFARI	NGMYNSPFFM
71	•	• • • • • • • • • • • • • • • • • • • •	•	•	NGSYNAPFFV
960	• • • • • • • • • • • • • • • • • • • •	• • • • • • • • • • • • • • • • • • • •	• • • • • • • • • • • • • • • • • • • •	•	. AYNAPPEV
19	•	TVLVNSPR	DAVLKLIRKI	NPNVFIPAIL	SGNYNAPFFV
Tfl	•	Ι	EKVLGVVKQD	TGDFHXWXRQ	EPNHNGPGFL
If	•	Ι	DKVLGVVNQI	KPEIFTVVEQ	ESNHNSPIFL
831	• • • • • • • • • • • • • • • • • • • •	NPWVPAPI	DILLKLVIKI	NPMI FTWVEH	EADHNRPPLL
S	•	SVSVEKYR	DRLLHLIKSL	SPNLVTLVEQ	EDNTNTSPLV
81	• • • • • • • • • • • • • • • • • • • •	SVTVENHR	DRLLRLVKHL	SPNVVTLVEQ	EANTNTAPFL
72	• • • • • • • • • • • • • • • • • • • •	SVCTENPR	DELLRRVKGL	KPRVVTLVEQ	EMNSNTAPFL
11	•	SVITVNOR	DELLHWYKSL	NPKLVTVVEQ	DVNTNTSPFF
-	•	•	•	•	•
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F16.150

	Wo	Motif VI			
•	GREVEAIHYY	SALPDSLGAS	Y. GEESEER	HVVEOOFTSK	STRAWT AVCC
8	QHFLNCFOSC	•	I. DADSA	CKTEDET TOD	DUFUNITA
m			•	Tarrent ace	
40	· C		THE STATE OF THE S	LAVERVLEGR	KIMDLVKSDD
	ו מ		L. DAM	QKIERFLIQP	EIEKLVLD
8	DRFTEALFYY	SAVFDSLDAA	N. NNNNNN	ORMEAEYLOR	EICHINGGEG
S	•	•			
9	ERLLESLYTY	AALFDCLETK	C	TKVFKM PCP	
11261	TRFREALFHY	SSLFDMFDTT	IHAEDEYKNR	STIFBELLVE	
7	P. F.	SATEDMETA	T DKUNEOD	TT TECAT FCB	Dana Amara
960	R	SSTEDMETT	V DRENEFE	WET EVENTED	B. ANVIOLEG
-			Vaadan 1	הוב הבחב עו פא	EALNVIACEG
7 6	2	SAVFUMCUSK	L AREDEMR	LMYVPEFYGR	EIVNVVASEG
	X	STXFDSLEGX	PNSQD	KLMSEXYLGX	QICNLVACEG
H	DRFTESLHYY	STLFDSLEGV	PSGOD	KVMSEVYLGK	OTCNVVACDG
	ERFTNALFHY	ATMFDSLEAM	HRCTSGRDIT	DSLTEVYLRG	ETEDIVCEEC
865	SRFVETLDYY	TAMFESIDAA	R. PRDDKOR	ISAEOHCVAR	DIVINATACER
81	PREVETMNHY	LAVFESIDVR	L. ARDHKER	INVEOHETAR	FUENT TACES
172	GRVSESCACY	GALLESVEST		AKVE FGTCD	KT UNAVACEO
11	PRFIEAYEYY	SAVFEST.DMT		Service Control	
	}		Valent in the second	THIN SENDENTE	DIVALVACEG
	•	. KXFDSLEHD	A. SKGEPRE	DERGRXCLAR	NIVNIVXCKX
	O				650

F16.15P

.NAAT OATLILGMFP		_	_			WE OARRITOGEG			•		-				KG	KS	NC	ES.	000
SLAG. N						PLSY. YAMI.	TISKOI	PLNODM	PFDPSI	_		HIGSNAF	WFDPDEVDTL	PVSTSAAFAA	PLSSYVNATI	PLSEKIAESM	PMSAKVTNNI	PMSGRVTSN	
KMOOCGFKGI	VFAATGFKPV	LMEKAGFEPV	MFLOMGFSPV	RLTRAGLSAV		RIDLAGFGNV	RILRAGFKPA	RNORVGFKOL	RAMRSGLVOV	RLIRAGFROL	RFGSSGLAPA	RFGSAGFAAA	RLTYAGLTOV	RMMAGFIGW	RFHMAGFKPY	RMSMAGFELM	RMMAGFNPK	RMMMAGFSPR	
ESWRE		LMEEKEQWRV		PLSRWRD	•	3	TYKOWRV	TYKOWOV	TYKOWHV	TYKOWQA	TLSQWGN	TLSOWRN	LFGHWRE	VLGKWRV	PLGKWRS	VFGKWRM	AAGKWRA	VIGKWRA	
PSRSGEVKF.	.RHKAQ	DNNKPGTRFG	RSRPIER.	AARXERHE	•	FERRERHE	AERFARPE	LERMERPE	WERVERPE	TERVESRE	PDRVERHE	PDRVERHE	SARTERHE	SERVERHE	VEREERHE	IDRIERCE	EERIERYE	EERIERYE	651
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Motif	.SDGYTLVD.	VRGFHVEK	YSTLYSLVES	VRGFH. VEE	•	•	FDGYR IKE	RYHRDFVIDS	LPTRTFIDE	FYHKDFVIDQ	GYDKNFDVDQ	NSGOCYRVEE	NGGEGYRVEE	. SGSGFNILV	AYDKNYKLGG	SYSEKYTLEE	VHPG. FTVKE	QYCNKYKLKE	DYCSKYKVKE	701
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SRPa1 (1110)

53/74

CTTTGTCAATGGTAAATGAGCTGAGGCAGATAGTTTCTATCCAAGGAGACCCTTCTCAGA Gaatcgcagcttacatggtggaaggtctagctgcaagaatggccgcttcaggaaaattca TCTACAGAGCATTGAAATGCAAAGAGCCTCCTTCGGATGAGAGGCTTGCAGCTATGCAAG TCTGTTTGAAGTCTGCCCTTGTTTCAAGTTCGGGTTTTTAGCAGCTAATGGTGCGATAC Ttgaagcaatcaaaggtgaagaagtacacataatcgatttcgatataaaccaaggga ACCAATACATGACACTGATACGAAGCATTGCTGAGTTGCCTGGTAAACGACCTCGCCTGA GGTTAACAGGAATTGATGACCCTGAATCAGTCCAACGCTCCATTGGAGGGCTAAGAATCA Taatagtgaactttgcattccaacttcaccacgtgcctgacgaggggtgcacaacagtaa **ACCAGCGGGACGAGCTACTTCACATGGTCAAAAGCTTAAAACCCAAAGCTTGTCACGGTCG** TOGGTCTAAGACTCGAGCAACTCGCAGAGGATAATGGAGTATCCTTCAAATTCAAAGCAA TGCCTTCAAAGACTTCGATTGTCTCCCATCAACACTCGGTTGCAAACCAGGAGAAACCT Tigaacaagacgtgaacacaaacacttcaccgttctttcccagattcatagaggcttacg CTGGATTCAATCCAAAACCAATGAGTGCTAAAGTAACCAACAATATACAAAACCTGATAA GGGAGGAGAAAAGCTTAATCGTTGCTTCAGCTTGGAGGTAAGATAAGTGACAAGAGCATA **Tagtctttatgtttcataaaacataattatgttttttactgtaatcttgggttattgtgta ACTGGTTAAATCATCTCCATGTATTATTACCAGAGGTTAGGGGTGATCACAGGTACTAAA AATTAGAGTTTTGGTTCTAAACCTATTTGCTAAGTGTGAATGAGTCTTTACATGTTCATA AGCAACAATATTGCAATAAGTACAAGCTTAAAGAAGAAATGGGTGAGCTCCATTTTTGCT**

F16. 16A

SRPa3 (3935)

TGGCTTGCTTTGCTTCAAGCTTTTAACTCTAGGCCTGAAGGTCCACCTCATTTGAGAATC GCTATGGAAGGAGAAGATGGTTCATGTGATTGATCTCGATGCTTCTGAGCCAGCTCAA **ACTGGTGTTCATCACCAGAAGGAAGTGCTTGAACAAATGGCTCATAGACTCATTGAGGAA** GCAGAGAAACTCGATATCCCGTTTCAGTTTAATCCCGTTGTGAGTAGGTTAGACTGTTTA GCTGAGGCACGTGAGAATGATATGAGTAACAACAATGGGTATAGCCCTAGCGGTGACTCG GCCTCATCTTTGCCTTTACCAAGTTCAGGAAGGACTGATAGCTTCCTCAATGCTATTTGG aatgtagaacagttgcgggttaaaacaggaggggccttagccgttagctcggttcttcaa TTGCATACCTTCTTGGCCTCTGATGATCTCATGAGAAGAACTGCGCTTTACGGTTT CAGAACAACCCTAGTGGAGTTGACTTGCAGAGAGTTCTAATGATGAGCCATGGCTCTGCA GGTTTGTCTCCAAAGGTCATGGTGGTCACTGAGCAAGACTCAGACCACAACGGCTCCACA CTAATGGAGAGGCTATTAGAATCACTTTACACCTACGCAGCATTGTTTGATTGCTTGGAA ACAAAAGTTCCAAGAACGTCTCAAGATAGGATCAAAGTGGAGAAGATGCTCTTCGGGGAG GAGAAATGGAGCCAGAGGATCGATTTGGCTGGTTTTGGGAATGTTCCTCTTAGCTATTAT GCGATGTTGCAGGCTAGGAGATTGCTTCAAGGGTGCGGTTTTGATGGGTATAGAATCAAG TGGAGATGCAGGAAGTGAATGATATATTACAGTTTGTCTTCTATTTTGGTTATGAGCAGA **CTGTCTCTTTATGCTATTTTGGCTTAAATGCTTCTACTGCCTCTGCATGTAAAGCCTTTG** GAAGAGAGCGGGTGCGCAGTAATTTGCTGGCAAGATCGACCTCTATACTCGGTATCAGCT TGTGTTGGTTCAATTTGGTCTGGTGTGTGTAATACCAAACCAAATCCAATTTGAGCTG GTCCCTTTCTTTTTGTATACATGGGGACACAATCTTAGTTGTTTTGTGATGGTGACTTT **AAGATAACTAATTTGATGATCGGCTCGTGCC**

F16, 16E

SRPa4 (4818

GGCACGAGCCCAACGGGTCCTGAGCTTCTTACTTATGCATATCTTGTATGAAGCCTGC **CCTTATTTCAAATTCGGTTATGAATCTGCTAATGGAGCTATAGCTGAAGCTGTGAAGAAC** GAAAGTTTTGTGCACATTATCGATTTCCAGATTTCTCAAGGTGGTCAATGGGTGAGTTTG **ATCCGTGCTCTTGGTGCTAGACCTGGTGGACCTCCGAACGTTAGGATAACGGAATTGAT** AAGCTAGCTGAAATGTGCGGTGTTCCGTTTGAGTTCCATGGAGCTGCTTTATGCTGCACG SAAGTCGAAATCGAGAAGCTAGGAGTTAGAAATGGAGAAGCGCTCGCGGTTAACTTCCCG **TTGAGATTGGTCAAACACTTGTCACCAAACGTTGTGACTCTGGTTGAGCAAGAAGCGAAT FTCGAATCAATAGATGTGAAACTCGCTAGAGTCACAAGGAAAGGATCAATGTTGAGCAG** CATTGTTTGGCTAGAGGTTGTGAATCTTATAGCTTGTGAAGGTGTTGAAAGAGAAGAG **AGGCACGAGCCACTAGGGAAATGGAGGTCTCGGTTTCACATGGCGGGATTTAAACCGTAT CCTTTGAGCTCGTATGTGAACGCAACAATCAAAGGATTGCTTGAGAGTTATTCAGAGAAG** TATACACTTGAAGAAGAGATGGAGCATTGTATTTAGGATGGAAGAATCAACCTCTTATC **ACTICTIGTGCTTGGAGGTAACTAATAAAACCTTGTTCGGTTTCAGAAGAGATTAGAAA** ACAAACACTGCGCCGTTTCTTCCCCGGTTTGTCGAGACAATGAACCATTACTTGGCAGTT

F1G. 16C

SRPa5 (4871)

TTCCATGGCTTCCACGTCTCCACATCATCGACTTCGATATCGGCTACGGTGGCCAATGG **CTCAAGCACTTCGCCTCTGAGATCAACATCTCCCTTGACATCCAAGTTTTGAGCTTAGAC** GCGGCTATCTTCTACGCCCACCACCATACACCTCCGCCGGCAAAGCGGCTCAACCCT GGTCCCGTGGGGATAACAGAGCAGCTGGTTAAGGCAGCAGAGGTCATAGAGAGCGACACG TGTCTAGCTCAGGGGATATTGGCGCGCTCAATCAACAGCTCTCTTCTCCCGTCGGGAAG TCCCAAACCCTAAACCCTTATTCCCTCATCTTCAAGATCGCTGCTTACAAATCCTTCTCA GCTTCCCTCATGCAAGAGCTTGTTCTCCGCGACAACGCCGCTCCTCTCTCCCTCAAGATC **ACCGTTTTCGCTTCTCCGGCGAACCACGACCAGCTCGAACTTGGCTTCACTCAAGACAAC** CTCCTCGGCTCCATCTCGTGGCCTAACTCGTCGGAGAAGAAGAAGCTGTCGCCGTTAACATC **CCATTAGAAAGAGCAGCTTTTTACTTCAAAGAAGCTCTCAATAATCTCCTTCACAACGTC** ACGATCATCGTCTGCTCCGACAGAGGATGCGAGAGGACGGATCTGCCCTTCTCTCAACAG CTCGCCCACTCGCTGCACTCACACCGCTCTTCGAATCCCTCGACGCCGTCAACGCC **AACCTCGACGCAATGCAGAAGATCGAGAGGTTTCTTATACAGCCGGAGATAGAGAAGCTG** GTGTTGGATCGTAGCCGTCCGATAGAAAGGCCGATGATGACGTGGCAAGCGATGTTTCTA **CCACCGGAGTTTCAATTTTAAAAAAAAATATTTTCCTTAATTCAATTTTATCTTAAATGACA AATTTTTAGTTTTCTGATTTTTATTTTGCTCAGTGCGATGGATTTTTAAATTTTAAGTTTCAC** GTCCAACGGACGCCAGTGAGAGGCTTTCACGTCGAGAAGAAACATAACTCACTTCTCCTA CAGATGGGTTTCTCACCGGTGACGCACAGTAACTTCACGGAGTCTCAAGCCGAGTGTTTA TGTTGGCAAAGGACAGAACTCGTCGGAGTTTCAGCATGGAGATGTCGCTCCTCCTGATTT **ACAAATATATAAATTTTTG**

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SRPa6 (12398)

TGTTCGGTAGGAGGATTATGGATTTGGTCCGATCAGATGATGATAATAAACCGGGAA CCCGGTTTGGGTTAATGGAGGAGAAGAACAATGGAGAGTGTTGATGGAGAAAGCTGGAT ACAATTATAGTACATTGTATTCACTTGTTGAATCGGAGCCAGGTTTCATCTCCTTGGCTT aatctagtatttgagttagcttttagaattgaattgtttggggttagatttggatgtt **AATTAGTCTCTAGCCTATTCTCTTACTCTTTTTTTGTCTAGTGCTTGGAGTGATGATGGTT** TTGAGCCGGTTAAACCGAGTAATTACGCGGTTAGCCAAGCGAAGCTGCTACTATGGAACT GGAACAATGTGCCTCTCCCCGTTTCCTCTTGGCGTTGACTACTTGGTCCGATAAGTT AAAAAAA

F16.16

SRPa7 (21729/3635/17410)

AAAGACTTTAGCAGATTTTCAAGCGGCTCAGAACATCAACAACAACAACAACAACAACCA GTTTGGTGGTTCTGGTTCTCAGCGTTACGGTTTACCGGTTCCCAGGTCTCAGACGCA **ACAGCAACAATCGGATTACGGTTTATTTGGTGGGATCCGAATGGGAATCGGGTCGGGTAT TAATAATTATCCAACATTAACCGGCGTTCCGTGTATTGAACCGGTTCAAAACCGGGTTCA TCAAAATCTCGTGACTCCGAACCCGAACCCGGTTTTGTCTTTTTCACCGAGCTC TTCTTCTTCGTCTTCTTCTACAGCTTCGACGACGACATCGGTATGTTCTAGGCA** TTTTATAGTCAAGCAGCTCTCAACGCTTTTCTTTCAAGGTCTGTGAAGCCTCGAAATTAT TGAATCGGAGAACATGTTGAATAGTTTAAGAGAGCTTGAGAAACAGCTTTTAGATGATGA CGATGAGAGTGGTGATGATGACGTGTCAGTTATAACAAATTCAAATTCCGATTGGAT **AACGGTTATGGAAATCGCGACGGCGATCGCGGAAGGGGAAAACAGAGATAGCGACGGAGAT TTGGCGCGTGTTTCTCAAACGCCTAATCTTGAGAGGAATTCAGAGGAGAAGCTTGTTGA** CEAGGCCGCGAATCTCGCCATTCTCGACGCCGCCGATAACAACGAGGGTGGAATGATGAT CAGAATTTTCAATCTCCGTCGGCCGATGATTGATCTCACGTCGGTGAATGATATGAGTTT **TTTCATGGTGGCTGCGCTTCGATCGAGGATAGCTTCTCCAGTGACGGAATTGTATGGGAA ACCGCACGTTATCGATTTCGATATCGGAGAGGTGGACAATACGTTAACCTTCTCCGTAC** GGAGCATTTAATCTCGACTCAATTGCTCTACGAGCTCTCTCCTTGTTTCAAACTCGGTTT **ATTATCCACGCGCGGAATGGTAAAAGTCAGAGTCAGAATTCTCCGGTGGTTAAGATCAC AGCCGTCGGAGATTTGTTGAGCCAACTCGGTGATCGACTCGGTATCTCCGTAAGTTTCAA** CGTGGTGACGAGTTTACGACTCGGTGATCTGAATCGTGAATCTCTCGGGTGTGATCCCGA CEAGACTTTGGCTGTGAACTTAGCTTTCAAGCTTTATCGTGTTCCCGACGAAAGCGTATG CACGGAGAATCCAAGAGACGAACTTCTCCGGCGCGTGAAGGGACTTAAACCGCGCGTGGT GTCATGCGCGTGTTACGGTGCGTTGCTTGAGTCGGTCGAGTCTACGGTTCCTAGTACGAA **TTCCGACCGTGCCAAAGTTGAGGAAGGAATTGGCCGGAAGCTAGTAAACGCGGTGGCGTG** CGAAGGAATCGATCGTATAGAGCGGTGCGAGGTGTTCGGGAAATGGCGAATGCGGATGAG CATGGCTGGGTTTGAGTTAATGCCATTGAGTGAGAAGATAGCGGAGTCGATGAAGAGTCG

1.16 16F-1

TTTCTTCTTATTACCATATTATTATTAATTTTCGAGATTATTCTGATATTATTATCA TTGTGATTTTCCGTTTCGAAAAGTĠTAGGAATCTTATGTAACAAAGAAAAAAAAAGACT TTGGATGGGACGGCACTCACTGTCGCATCCGCTTGGCGTTAACTTCACACACTCTTTTT AAAAAAA

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SRPa8 (10964)

TGCATACAACGCACCGTTTTTCGTAACACGGTTTCGCGAAGCTCTATTTCATTTCTCCTC GATTTTTGACATGCTTGAGACAATTGTGCCACGAGAAGAGGAGGAGGATGTTCCTTGA GGAGAGGCCTGAGACATACAAGCAGTGGCACGTACGGGCTATGAGGTCAGGGTTGGTGCA GGTTCCATTTGACCCAAGCATTATGAAGACATCGCTGCATAAGGTCCACACATTCTACCA GCATATGAGAGACCATCTCTTGATTTTCTTCCTGTGTAATTCCCAGAGACAGAATTACAG **ATGTAAGAAGAATGCTGCACAAAGAACTTGTTCAAAGATAATATTGATGTAAGTCCTG** Caaggattttgtgatcgatcaagataaccggtggctcttgcaaggctggaagggaagaac TGTCATGGCTCTTTCTGTTTGGAAACCAGAGTCCAAGGCTTGACCGAGAAATCCTCGTTG TTTTATAACTTTCTAGCTGTGTTTTTGTTGTTTCTCAGCTAGATTCTCCTAACGGTATTC Taacacatataagaggaagcttagagtttctatgggtttaaagagaagttttttccttctc Caatgtaaaaaaaaaaaaaaa

F16.160

SRPa10 (11261

AGGAAACGTTACCATAGAGATTTTGTGATCGATAGCGATAACAATTGGATGCTTCAAGGA aaaaaatgggaaaccatcactcttgatgaacttatgatcaatccaggagagacaacggtc GTCAACTGCATTCATCGGTTACAATACACTCCTGATGAAACTGTGTGTCATTAGACTCTCCA AGAGACACGGTTCTGAAGCTATTCAGAGATATCAATCCTGACCTCTTTGTGTTTGCAGAG **TACTCTTCACTCTTTGACATGTTTGACACCACAATACACGCAGAGGATGAGTACAAAAAC AGGTCACTGTTGGAGAGAGTTACTTGTGAGAGACGCGATGAGCGTGATTTCCTGCGAG** GGTGCAGAGCGGTTTGCGAGGCCTGAAACCTACAAGCAATGGCGAGTTAGGATTTTGAGA GCCGGGTTTAAGCCAGCAACTATTAGCAAACAGATCATGAAGGAGGCTAAGGAAATTGTG TGGAAAGGAAGAGTCATCTATGCTTTTTCTTGCTGGAAACCTGCTGAGAAGTTCACAAAC **ITTTGTAGAATATGTTTGATCCCGTGAGTGGATGCAACTCTTTTTTCCTGCAAGTACATA** ATTAACGGAATGTACAACTCTCCTTTCTTCATGACGAGGTTCCGAGAAGCGCTTTTTCAT

F16.16

SRPa11 (18652)

GAACCTGGCTTTGCTGTTGTGAACTTCCCATATGTATTACACCACATGCCAGACGAG GGGCCGCCGTTGCTGCGTGTGACGGGTGTGGATGATTCACAGTCCACCTATGCTCGTGGG GGAGGACTCAGCTTGGTAGGTGAGAGGCTTGCAACTTTGGCGCAGTCATGTGGTGTCCCG TTTGAGTTTCACGATGCCATCATGTCTGGGTGCAAGGTGCAGCGGGAACATCTCGGGTTG **AGCGTAAGTGTTGAAAATACAGAGACAGGCTGCTGCATCTGATCAAGAGCCTCTCCCCA AAACTGGTTACTCTAGTAGAGCAAGAATCCAACACAAACACCTCGCCATTGGTGTCACGG** CGGGATGATAAGCAGAGAATCAGCGCAGAACAACACTGTGTAGCAAGAGACATAGTGAAC ATGATAGCATGTGAGGAGTCAGAGAGAGTAGAGACACGAGGTACTGGGGAAATGGAGG CAGATTGCACAGGGATCACAATACATGTTTTGATTCAGGAGCTTGCGAAACGCCTGGT TTTGTGGAAACACTGGATTACTACACAGCGATGTTTGAGTCGATAGATGCAGCACGGCCA GTCAGAATGATGATGGCTGGGTTCACGGGTTGGCCGGTCAGCACATCTGCAGCGTTTGCA GCGAGTGAGATGCTGAAAGCTTATGACAAAAACTACAAACTGGGAGGCCATGAAGGAGCG CTCTACCTCTTCTGGAAGAGACGACCCATGGCTACATGTTCCGTGTGGAAGCCAAACCCA GCGAATGTTGAGATCTTGGAAGCAATAGCTGGGGAAACCAGAGTCCACATTATCGATTTT **AACTATATTGGGTAAGTTATAGTGATGATGGTTACTTGAGTGGATAAAGAAGAGCACAAC AAAAACACATCTGTCGCTGTAAATTTTTTAGGATGTGCAATGATGTTTTAAGTTGTAACA CAACCTAAGTTATATATGTATACAAACCAAACCTGGTGGTTGTTTTTTCTCTTGTAAATTG** TCATGTGGTTGGGTGGGAAGCTAGTAATGAAATATAACCAAAACATTGATTAGGTCAA AAAAAAAAAAA

F1G. 16I

16.16J-1

AGAAAGATGAGACAGAGCATCATCATCATAGCTATGCACCACCACCAACAGATTAA TCCACATAATAGATTTCGGAATATCTTACGGTTTTTCAGTGGCCTGCTCTGATTCATCGCC CACAAGCTGTATCAGTGGATGATCGTAGAACCGCCAACGTTTAGCTAAGGCAGATACGAG AGCATTCTTCGCCTCTAGGCAATGGTTCAGAGCGGTTGGCTCATTATTTTGCAAATAGTC TTGAAGCACGCTTAGCTGGGACCGGTACACAGATCTACACCGCTTTATCTTCGAAGAAAA **AAGCTGCTATCATATTTGCTAACCACAGCATGATGCGTTTCACTGCAAACGCCAACACGA** ACAGTGTCACCGGTGGTGGTGGTGGTAATAGTGCGGTTTACGGTTCAGGTTTTGGCG **ATGATTTGGTTTTCGAATATGTTTAAAGATGATGAATTGGCTATGCAGTTCAAGAAAGGGG** agcaatcagctgtttatgttgaggaaagcgagctttctgaaatgtttgataacatgttcc **TATGTGGCCCTGGGAAACCTGTATGCATTCTTAACCAGAACTTTCCTACAGAATCCGCTA AAGTCGTGACCGCACAGTCAAATGGAGCAAAGATTCGTGGGAAGAAATCAACTTCTACTA** GTCATAGTAACGATTCTAAGAAAGAAACTGCTGATTTGAGGACTCTTTTGGTGTTATGTG CTGGTAAGAAAGCCATTGGCGCGACGAGATGAAGATTTCGTTGAAGAAGAAGTAACA TTGAGGAAGCTAGTAAGTTCCTTCCTAAGTCTTCTCAGCTCTTTATTGATGTGGATAGTT

SRPa12 (23196)*

TCTTACTCAAGGTTCTTCTTTGTCTTGTTGCCGAATCCACAAAGAGGAGAATAAAGA TICGACCITITATIAGATATITAACGACICIGGATITITIGGGITITITGGAGTIGGATCCACA TGGGTTCTTATCCGGATGGATTCCCTGGATCCATGGACGAGTTGGATTTCAATAAGGACT TTGATTTGCCTCCTCCTCAAACCAAACCTTAGGTTTAGCTAATGGGTTCTATTTAGATG **ACAACATCAACAACAAAGCTGTAGCAGGAGATCTGTTATCATCTTCATCTGATGACGCTG** atttctctgattctgttttgaagtatataagccaagttcttatggaagagatatggaag **AGAAGCCTTGTATGTTTCATGATGCTTTTGGCTCTTCAAGCTGCTGAGAAATCTCTATG AGGCTCTTGGTGAGAAGACCCTTCTTCGTCTTCTGCTTCTTCTGTGGATCATCCTGAGA** GATTGGCTAGTCATAGCCCTGACGGTTCTTGTTCAGGTGGTGCTTTTAGTGATTACGCTA

GCACCACTACCACTACTTCCTCATTCTCACTGGAGTGTTGATGGTTTGGAGAATAGAC **CTICTIGGITACATACACCTATGCCGAGTAATTTTGTTTTCCAGTCTACTTCTAGGTCCA** NNNNNNNNNNNNNNNNNNNNNNGAGTTCAGGAGACAGGTCATCGCTTGGCTCGATACT GTCAGCGACACATGTTCCGTTTGAGTACAACGCAATTGCTCAGAAATGGGGAAACGATC CAAGTCGAAGACTTAAAGCTTCGACAAGGAGAGTATGTGGTTGTGAACTCTTTGTTCGT TTCAGGAACCTTCTAGATGAGACCGTTCTGGTAAACAGCCCGAGAGATGCAGTTTTGAAG CTGATAAGAAAATAAACCCGAATGTCTTCATTCCAGCGATCTTAAGCGGGAATTACAAC GCGCCATTCTTTGTCACGAGGTTCAGAGAAGCGTTGTTTCATTACTCGGCTGTGTTTGAT **ATGTGTGACTCGAAGCTAGCTAGGGAAGACGAGATGAGGCTGATGTGTGTTTGAGTTT SAGACATATAAGCAGTGGCAGGCGAGACTGATCCGAGCCGGATTTAGACAGCTTCCGCTT** GAGAAGGAACTGATGCAGAATCTGAAGTTGAAAATCGAAAACGGGTACGATAAAACTTC **SATGTTGATCAAAACGGTAACTGGTTACTTCAAGGGTGGAAAGGTAGAATCGTGTATGCT PCATCTCTATGGGTTCCTTCGTCTTCATAGATGTTGTTTCTTACGTTCTAAGCGACTGGG ATTTATGTAGGGCTTTTCTGTTGATAGTCTCTCGCCAACACGGGGTGGATTAAGTTCAGAG FTAGGGTTCTTGAACACTAGAATGTTGTTATATTATGCTTGTGACATAGCGTGTGAAGA** STGTAGCCTAAGAGATATAGTACTCATTGCATGATCTTTTGCTATATGTTNCATGT

F1G. 16J-2

SRPd1

TCTGCAGACAATTTTNAGGAGGCCAATACCATGCTATTGGAAATTTCAGAACTG AATGTCGGNNAGATTAGTTAGCTCCTGCTTAGGAATCTATGCTTCTCTTCCNGC TCCACACCTNNNNNNNNNNNNNNNNNNNNNNNNNNNNNGTACTTCTCAGAGGN AACAGTGGTGCTCCTCATGGTCAGAAAGTGGCCTCA

F1G. 16K

SRPq1

TCAACTGAGAATCTAGAAGATGCCAACAAGATGCTTCTGGAGATTTCTCAGTTA TCAACACCGTTCNNCACTTCAGCACAGGGTGTGGCAGCATATTTCTCAGAAGCC ATATCAGCAAGGTTGGTGAGTTCATGTCTAGGGATATACGCAACTTTGCCACAC **ACACACCAAAGCCACAAGGTAGCTTCAGCTTTTCAAGTGTTCAATGGTATTAGT** CCTTTAGTGGAGTTCTCACACTTCACAGCAAACCAAGCAATTCAAGAAGCCTTC GAAAGAGAAGAGGGTGCACATCATAGATCTTGATATAATGCAAGGGTTG

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TCTGCAGACAACTTTGAAGAAGCCAATACAATACTGCCTCAGATCACAGAACTC TCCACCCCTAINGCAACTCGGTGCAACGAGTGGCTGCCTAINNNNNNNNNNN

SRPp1

ATTCACATGTCCCAGAGCCAGAAAATTGTGAAT

1G.17A-1

AAACGCCCCAACCAGAGCATCCACGTCTCTTTTTCACCTTCATCGTTGGATCATAGA TGAGCGGTCCACGGTGAACTCCGTTTGCCTGCAAAACCACGTCCTCTACGCGCTGTTAAG TAGCTTCTAGAAACATCACGATGTGTCCCGTCCATTCCTTTAGGAGGAGCCGGATCCGGC GGAGGTGCAGCGGGAAGCAGCGCGACGAGGAGGCCCTCCACCTGCTGAGTGCTGACGC GCCGCAGTCGCCCAAGGTCCCGACCGCCGCGCCTCGGCCGCCGCCGCCAAGGAGCGGAA TGCTGCTGCAGTGCGCGGAGGCCGTGAACGCGGACAACCTCGACGACGCGCACCAGACGC **ACTTCGCGGAGGCCATGTCGGCGCGCGTCAGCTCCTGCCTAGGCCTGTACGCGCCGC** TGCCGCCGGGCTCCCCCGCGCGCGCGCCTCCACGGCCGCGTGGCCGCCGCGTTCCAGG TGCTGGAGATCGCGGAGCTGGCCACGCCGTTCGGCACCTCGACCCAGCGCGTGGCCGCCT TGTTCAACGGCATCAGCCCCTTCGTCAAGTTCTCGCACTTCACCGCCAACCAGGCCATCC **AGGAGGCGTTCGAGCGGGAGGAGCGTGTGCACATCATCGACCTCGACATCATGCAGGGGC** GGCTCACCGGCCTGGGGGCGTCCATGGACGCGCTCGAGGCGACGGGGAAGCGCCTCTCCG **ACGTIGACCCGCAGAAGCTGGGCGTCACGCGGCGGGAGGCCGTCGCCGTCCACTGGCCGC ACTTCGCCGACACGCTCGGCCTGCCCTTCGAGTTCTGCGCCGTCGCCGAGAAGGCCGGCA** ACCACTCGCTTTACGACGTCATCGGCTCCGACTCCAACACGCTCTGGCTCATCCAAAGGT **CCTCCATTTTCCTTCTCCCTTTCTTCCATGTCAAATCTTGATGCAATCATGACCACTT TTCAGCTGCTGACATTGGATAATGTGAGCTTTACGGCAAGCATCAAGTCGTGGTAGTACA**

Partial DNA sequence of ZCARECROW gene

AATTTGGCCACAAGGTATGACACTGTCTCAATTGAGCAATCTAGTAGAGAAACTGATCCA TCATATATTGCTCATATTGAAAGTGAAAAGATATGCTCAAGAACCTAGTAGAGAAGCTA **Aaaattgaaaaatctagctctactagaaaaatatgataggttgcctgtttctcatgaaa** TTTATTAGATAATCATATCATGGCTAGATGTCGCTCATGAGGTTGTTCTTGCTAGTTTAG **ATTCCTGTGGGCATTCATCTCTTTAGATGCACTAACATGATAGGAAGTTTCTAATCTGG** TGCTTCACAATTCTGGTGATTCATGCTTCCTTCATTGCAATTGATATTGATGCTTGATTC

gatatcagcatcattttaaatgtaagttggcaaaagatcatgagggttctcatagt

CTGCAACTAGTGTTTCACCATGTGGTTTTTTAGTATCATTCGTATTAGTTTTCTAACTTTC

AACTIGITITIAGCGIGTACGTTAGCATTATAATATTTCCTTATTATGAAAGCGGAAGAG

TATTGATATATAAAGTGATAACTAGTTTTAGAAATATTCTCTTGTGCCATTAATGCTAC

CGCGTTTTGAAGCTCAAAAGGCGATTTCTTCCGAGGTTTGCTGTTGAGCGCTATTTTGGA TGAAATGGAGTCCCAAACTAACCCTAATATAAAAAAAAAGGCGCTTTGGAGCTCAAAACG TGAAAAGGTTGAATCTTGACAATGCTTTTGAGATGATACTGTAGTGTAGTCTGTAGTGGA CTCGTTGTTATGACCAACCAGCTTTATAGGTTTAAAAAGGTTGAATCTTGACAATGCTTT GCATCCTCCATGGTCTTTGGTGATCGAGAATTCCTGCAGCCCGGGGGATCC

TCCATTACAGCTATTTCTAAAATATTCTTCGGAGGTTTCCTGCTCATAGTAAAAAAA

F16.17A-2

protein **ZCARECROW** Partial amino acid sequence of

LHNSGDSCFLHCNXYXCLIHASVTLCVXLVLYVSLDCRVSATSVSPCGFLVSFVLVSNFL YQHHQFXMXVGKRSXGFSXXFGHKVXHCLNXAIXXRNXSIIYCSYXKXKRYAQEPSREAK NXKIXLYXKNMIGCLFLMKIYXIIISWLDVAHEVVLASLDSCGHSSLLDALTXXEVSNLV LIYXSDNXFXKYSLVPLMLQLVFSVYVSIIIFPYYESGRETRPTRASTSSHFTFIVGSXM SGPRXTPFACKTISSTRCXVASRNITMCPVHSFRRSRIRRRSRPRSRPPRPPPRSGR RCSGGSSATRRASTCXV<u>LTLLLQCAEAVNADNLDDAHQTLLEIAELATPFGTSTORVAAY</u> FAEAMSARVVSSCLGLYAPLPPGSPAAARLHGRVAAAFOVFNGISPFVKFSHFTANQAIO EAFEREERVHIIDLDIMOGLOWPGLFHILVSRPGGPPRVRLTGLGASMDALEATGKRLSD FADTLGLPFEFCAVAEKAGNVDPOKLGVTRREAVAVHWPHHSLYDVIGSDSNTLWLIORS SIFLLCLSSMSNLDAIMTTFQLLTLDNVSFTASIKSWXYIHYSYFXNILRRFPAHSKKKS RFEAQKAISSEVCCXALFWKPHFLNXFLFFKEKLVHFSLVKWSPKLTLILKKTRFGAQNA RCYDQPALXVXKGXILTMLLKRLNLDNAFEMILXCSLXWSILHGLWXSRIPAARGI

F16.17B

065	YAALPSRWMPOTH-SLKWVSA FOVFNGT CDT VICEGUEMANGA TOTAL	YASLPATVVPPHGQKVAS	YATLPHTHOSHKVASAFOVFNGISPLVEFSHFTANOATOFAEF	YSPLPPIXMSQSQKIVN
		SRPd1		

412	OLDIMOGT,	T.DTMOCT
397 412	KEDSVHIIL	REERVHTTE
	SCR	SRPq1

F 16. 18



FIG.19A



FIG. 19C



FIG.19B



FIG.19D



FIG.19E



FIG.19F

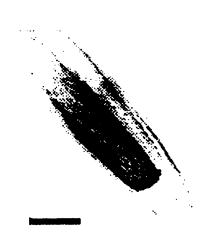


FIG.19G

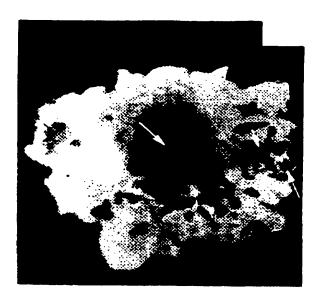


FIG.20A



FIG.20B

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WO 97/41152

SCR Promoter::GUS 73/74 SCR Promoter::SCR



FIG.21A



FIG.21B

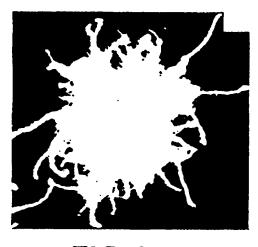


FIG.21C



FIG.21D



FIG.21E



FIG.21F

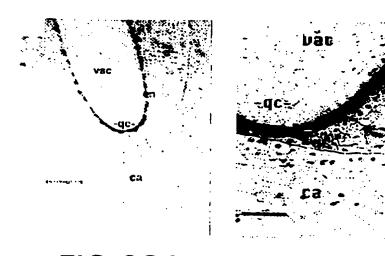


FIG.22A

FIG.22B

INTERNATIONAL SEARCH REPORT

International application N . PCT/US97/07022

A. CLASSIFICATION OF SUBJECT MATTER IPC(6) :C07K 14/415; C12N 1/21, 5/10, 15/29, 15/63; A01H 5/00									
US CL: Please See Extra Sheet. According to International Patent Classification (IPC) or to both national classification and IPC									
B. FIELDS SEARCHED									
Minimum documentation searched (classification system followed by classification symbols)									
U.S. : 536/23.6, 23.1; 435/320.1, 252.3, 419; 530/350, 370, 387.9; 800/205									
Documentat	tion searched other than minimum documentation to th	ne extent that such documents are included	in the fields searched						
	lata base consulted during the international search (or ALOG - Biotech Files, GenEMBL sequence data	•	, scarch terms used)						
C. DOCUMENTS CONSIDERED TO BE RELEVANT									
Category	Citation of document, with indication, where a	ppropriate, of the relevant passages	Relevant to claim N .						
Y	SCHERES et al. Mutations affecting the radial organisation of the <i>Arabidopsis</i> root display specific defects throughout the embryonic axis. Development. 1995, Vol.121, pages 53-62, see entire document.								
Υ, Ρ	WYSOCKA-DILLER et al. Root Physiology. June 1996, Vol. 1 40001, page 12, see entire abstra	111, No. 2, abstract no.	1-28						
Purth	er documents are listed in the continuation of Box (See patent family annex.							
	soial categories of cited documents:	T later document published after the inte	mational filips data as ariasta.						
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<u>the</u>	rement published prior to the international filing date but later then priority date claimed	"A" document member of the same points	heály						
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Box PCT Washington	, D.C. 20231	ELIZABETH C. KEMMERER	100th						
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romn PC 171S	A/210 (second sheet)(July 1992)*		\						

INTERNATIONAL SEARCH REPORT

International application No. PCT/US97/07022

A. CLASSIFICATION OF SUBJECT MATTER: US CL :										
536/23.6, 23.1; 435/320.1, 252.3, 419; 530/350, 370, 387.9; 800/205										